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Comparative susceptibility of three species of char and of rainbow trout × char triploid hybrids to several pathogenic salmonid viruses

Michel Dorson[1], Bernard Chevassus[2], Corinne Torhy[1]

[1] Unité Virus des Poissons, Laboratoire de Virologie et d'Immunologie Moléculaires, INRA, F-78352 Jouy-en-Josas Cedex, France

[2] Laboratoire de Génétique des Poissons, INRA, F-78352 Jouy-en-Josas Cedex, France

ABSTRACT: Triploid hybrids were obtained from rainbow trout *Oncorhynchus mykiss* females crossed with sires of brook trout *Salvelinus fontinalis*, arctic char *S. alpinus* and lake trout *S. namaycush* following heat-shock-provoked retention of the second polar body. Survival rates of hybrids obtained were ca 50 %. The hybrids and the parental species were challenged with infectious pancreatic necrosis virus (IPNV) type Sp, viral haemorrhagic septicaemia virus (VHSV) types 1 and 3, and infectious haematopoietic necrosis virus (IHNV). Rainbow trout × coho salmon (*O. kisutch*) triploid hybrids were included in certain infection trials. The results indicated that brook trout were resistant to VHSV, and brook trout × rainbow trout hybrids were resistant to both VHSV and IHNV, but susceptible to IPNV Arctic char were susceptible to IPNV and resistant to VHSV The hybrids were susceptible to IPNV, but partially resistant to VHSV Lake trout were partially resistant to IPNV, but their hybrids with rainbow trout were susceptible. Lake trout infected by VHSV showed clinical signs, and significant losses resulted.

INTRODUCTION

Rainbow trout *Oncorhynchus mykiss* is an important salmonid species reared in fresh water, especially in Europe, but its susceptibility to different viruses is an important limiting factor. A birnavirus, infectious pancreatic necrosis virus (IPNV), which affects young salmonids (Wolf et al. 1960), is spread worldwide (Wolf 1988), and a rhabdovirus, viral haemorrhagic septicaemia virus (VHSV) or Egtved virus, has plagued trout farming in continental Europe since 1950 (Jensen 1965, Wolf 1988). A second rhabdovirus disease previously restricted to North America and Japan, infectious haematopoietic necrosis (IHN) (Amend et al. 1969, Wolf 1988) has now been found in Europe (de Kinkelin et al. 1987). The occurrence of IHNV in Europe demonstrates the failure of sanitary procedures and regulatory controls in preventing the introduction and spread of viral pathogens, and although vaccines can be used in practice against VHS and IHN (de Kinkelin 1988, Leong et al. 1988) difficulties associated with their production, licensing and marketing have to date prevented their use in a commercial setting. In addition genetic approaches have also been considered (Chevassus & Dorson 1990).

Coho salmon *Oncorhynchus kisutch* are considered to be resistant to IPNV, VHSV and IHNV (Wolf 1988). Ord et al. (1976) demonstrated that the resistance to VHSV found in coho salmon was transmitted to hybrids obtained by conventional artificial fertilization between this species and rainbow trout. Unfortunately, the very low viability of such hybrids prohibited their practical use. The interest in hybrids increased when induction of triploidy in salmonids became routine and when it was shown that it unexpectedly improved the viability of hybrids (Chevassus et al. 1983). Triploid hybrids between rainbow trout females and coho salmon males proved resistant to VHSV (Dorson & Chevassus 1985) and to IHNV (Parsons et al. 1986). Unfortunately, these hybrids were susceptible to IPNV (Dorson & Chevassus 1985) as were diploid hybrids (Dorson unpubl. results), and it became interesting to examine other salmonid species. This is especially important because coho salmon are not commonly reared in Europe and are prohibited in several countries (e.g. France). In contrast, brook trout *Salvelinus*

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fontinalis is an acclimatized species commonly reared for restocking. Although the susceptibility of this species to IPN is well known, there have been no reports of its susceptibility to VHS, except an early and questionable report by Rasmussen (1965). Two additional species of char are reared in a few farms: arctic char *Salvelinus alpinus*, considered as aboriginal in France, and lake trout *Salvelinus namaycush*, which have become established in several alpine lakes. Because little was known about the possibility of triploid hybridization of these 2 species with rainbow trout, or about their susceptibility to viruses, except for a report by Silim et al. (1982) indicating the low susceptibility of lake trout to IPNV, we began research directed towards determining the susceptibility and potential use of hybrids created from rainbow trout and these 3 species of char. Initial studies involved only IPNV and VHSV and a comparison was made with rainbow trout × coho salmon hybrids. When IHNV began to spread in Europe, this virus was introduced in the experimental design.

MATERIALS AND METHODS

Brood stock. Rainbow trout *Oncorhynchus mykiss* used in the crosses and as controls came from the INRA trout farm of Gournay-sur-Aronde (Oise), France, where a 'synthetic strain' has been obtained by crossing different populations. A second source of rainbow trout ova was the domanial trout farm of Neuville-Ste-Gemme (Marne). Brown trout *Salmo trutta* were also obtained from Neuville-Ste-Gemme. Brook trout *Salvelinus fontinalis* were initially obtained from M. Jouy trout farm, at Avenay-Val d'Or (Marne), and later maintained at Gournay-sur-Aronde. Arctic char *Salvelinus alpinus* eggs and milt from wild spawners captured on spawning areas were provided by Dr C. Gillet at the INRA Station d'Hydrobiologie Lacustre of Thonon-les-Bains (Haute-Savoie). Lake trout *Salvelinus namaycush* eggs were obtained from the trout farm 'Les Sources Bleues', Thorens-Glières (Haute-Savoie), and later the fish were reared at Gournay-sur-Aronde. Coho salmon *Oncorhynchus kisutch* males were obtained from different private trout farms in France.

Fertilization procedure. Rainbow trout eggs were obtained by abdominal pressure from each individual female (from 3 to 7 depending on experiment), separated from coelomic fluid and distributed equally among different fertilization groups. Milt from different males (from 5 to 50) was pooled and the motility of sperm was observed by microscopy following the addition of 1 drop of buffered saline diluent (Billard 1977) to 1 drop of milt. Milt was always used in excess (1 to 5 ml per 100 g of eggs), and poured onto the eggs just before the diluent (10 °C, 50 ml per 100 g of eggs) was added. The gametes were mixed by 2 transfers between plastic bowls. Ten minutes after addition of milt, the eggs were gently rinsed and kept in flowing water (10 °C) to allow activation and hardening. At least 45 min later the eggs were disinfected in an iodophor (Romeiod, 50 mg l^{-1} available iodine, 15 min) and incubated in 10 l aquaria supplied with flowing tap water at 10 °C or in small incubators in an incubation recirculating unit also kept at 10 ± 0.5 °C.

Thermal shock was performed according to the procedure described by Chevassus et al. (1983). Thirty-five minutes after fertilization, the eggs were placed at 26.5 ± 0.1 °C for 20 min, returned to water at 10 °C for an additional 45 min, then disinfected with iodophor. Dead eggs were removed the next day and at the eyed stage. Mortalities were recorded daily, or at least twice a week. Survival of fry was assessed when all fish were actively feeding.

Rearing of fish. The eggs hatched in the 10 l aquaria. Although hatching curves were not established, the appearance of the first hatched fry and the times when 50 % and 100 % of fry had hatched were recorded. The age of a given group of fish was calculated from the day when ca 50 % were hatched. Resorption of yolk, start of feeding, and growth up to 5 g occurred in these aquaria. Afterward, the fish were reared in 60 l plastic tanks in recirculated filtered water. The fish were fed a commercially prepared feed (Gheerbrant).

Virus origin and production. The IPNV strain 31-75 was isolated in 1975 in Brittany from a severe outbreak of the disease in rainbow trout fry. The virus was later characterized by Dorson et al. (1978) and determined to be an Sp type (Vestergard-Jorgensen & Kehlet 1971). Since then, the strain has been passed periodically in susceptible fry to maintain virulence. Infectious virus was prepared at 15 °C in RTG_2 cells (Wolf & Quimby 1962) grown in Stoker's medium that was buffered at pH 7.4 with 0.16 M Tris-HCl and supplemented with 2 % foetal calf serum and antibiotics (penicillin 100 IU ml^{-1}, streptomycin 0.1 mg ml^{-1} and kanamycin 0.1 mg ml^{-1}. The virus suspensions used for infection trials were obtained from extracts of moribund fry after 2 passages in RTG_2 cells.

VHSV strain 07-71 was isolated from diseased rainbow trout in Normandy, and determined as belonging to serotype 1 (Vestergard-Jorgensen 1972). It was propagated in EPC cells (Fijan et al. 1983) at 15 °C in the same medium as described for IPNV. VHSV strain 34-86 (serotype 1) was isolated from trout at a farm in Marne. VHSV strain 23-75, serotype 3 (de Kinkelin & Le Berre 1977), was isolated from diseased brown trout *Salmo trutta* obtained from a Normandy trout farm.

IHNV, isolate 32-87, was recovered from rainbow trout experiencing one of the first outbreaks of the

disease in France (de Kinkelin et al. 1987). This strain was compared to representatives of North American strains and shown to be related to type 1 (RB 76) by Arkush et al. (1989). IHNV was cultivated in EPC cells in the same manner used for VHSV. Aliquots of the 5 virus isolates were stored at −70°C.

Virus suspensions were titrated on monolayers of the appropriate cell line by the plaque technique under agarose, described by de Kinkelin & Scherrer (1970). Plaques were counted after 3 d at 15°C following staining with 0.025% neutral red (IPNV) for fixation with 10% formalin and 1% crystal-violet staining (VHSV and IHNV).

Infection and monitoring of fish. Replicate groups of 30 to 250 fish (usually 100) to be tested were distributed into 2 aquaria. The water flow was stopped and virus was added to 1 aquarium to a final concentration of 5×10^4 pfu ml^{-1}. The second replicate aquarium served as a non-infected control. The fish were kept for 3 h with aeration before the water flow was resumed (10 to 30 l h^{-1}). All experiments were performed at water temperatures between 9 and 12°C. Each experiment involved appropriate controls, especially diploid and/or triploid rainbow trout representing the half-sibs of the hybrids under test, or rainbow trout or brown trout of the same age (± 1 wk), reared in exactly the same conditions. Mortalities were recorded daily until a plateau was reached (2 mo for IPNV, 1 mo for VHSV and IHNV). The weight of fish at the time of infection trials was 0.2 to 0.3 g with IPNV and 2 to 10 g with VHSV or IHNV.

Whole moribund or dead fish (≤ 1 g) or head, kidney and spleen (fish > 1 g) were ground with mortar and pestle in the presence of sterile sand. The supernatant was collected after centrifugation (5000 × *g*, 15 min) and used to inoculate RTG_2 cells in 24 well-plates (3×10^5 cells $well^{-1}$). The cells were incubated at 14°C and observed daily for evidence of cytopathic effects.

Statistical analysis. The mortalities between species and hybrids were compared by Chi-square analysis. An estimate of mortalities due solely to viral challenge was obtained by subtracting the mortality in non-infected control groups from the infected groups. The assumption was that non-specific mortality was equivalent in both groups and that this was independent of the virus. This leads to an estimate of the specific mortality (Sm) as follows:

$$\text{Sm}\ (\%) = 1 - \frac{\text{No. of survivors in the infected group}}{\text{No. of survivors in the control group}}$$

The values for Sm could be submitted to Chi-square analysis. This approximation was used only in a few cases where non-specific mortalities occurred in controls. A significance level of 5% was used for all statistical comparisons.

RESULTS

Brook trout and rainbow trout × brook trout hybrids vs rainbow trout × coho salmon hybrids

Rainbow trout (RT) ova were fertilized with milt from brook trout (BT), coho salmon (CS) and rainbow trout sires, and survival of the different genotypes is given in Table 1. The RT × BT hybrids hatched approximately 3 d later and the RT × CS hybrids 6 d later than the diploid and triploid rainbow trout, a phenomenon previously described by Quillet et al. (1988b). When fry started feeding, 54.6% (RT × CS) and 56.2% (RT × BT) of the hybrids had survived, compared to 66.9 and 90.6% respectively for the triploid and diploid rainbow trout controls.

Table 1. Survival to first feeding of rainbow trout × brook trout (RT × BT) 3n hybrids compared to rainbow trout × coho salmon (RT × CS) 3n hybrids. The ova from each of 6 rainbow trout females were equally distributed into the 4 groups. The ova were fertilized with milt from 6 sires of the respective species

	RT × BT 3n hybrids	RT × CS 3n hybrids	RT 2n	RT 3n
Total no. of eggs	1862	1770	1838	1720
No. of fry at start of feeding	1047	967	1666	1151
Survival (%)	56.2	54.6	90.6	66.9
Survival (% of 2n RT)	62	60.2	100	73.8

Infection trials were performed when the fish were 590 degree × days old for IPNV and 1550 degree × days for VHSV 1 and 3. Following exposure to IPNV, 78% of the RT × BT and 65% of the RT × CS died, compared to 87 and 94% of the 2n RT and 3n RT respectively (Table 2). Following infection with VHSV 1 and 3 a very low mortality was recorded in brook trout and in both hybrids (from 4 to 7%) while mortality among VHSV-exposed rainbow trout ranged from 68 to 93%. The brown trout infected with $VHSV_3$ suffered a 56% mortality but, as expected, were totally resistant to $VHSV_1$.

The susceptibility of RT × BT hybrids obtained in the laboratory to challenge with $VHSV_1$ was examined for 5 yr following our initial experiment. The data from all studies are summarized in Table 3. $VHSV_3$ was used in only 1 additional test and the mortality among hybrids was ca 3-fold less than that found with rainbow trout. A second $VHSV_1$ type strain (34-86) used on one occasion caused 100% mortality in rainbow trout and only 18%

Table 2. Final mortality (%) in groups of rainbow trout × brook trout (RT × BT) and rainbow trout × coho salmon (RT × CS) 3n hybrids, 2n and 3n rainbow trout, brook trout and brown trout infected with IPNV, VHSV 1 and VHSV 3. For each genotype, left and right columns correspond to 2 different experiments

Virus	RT × BT 3n hybrids		RT × CS 3n hybrids		RT 2n		RT 3n		BT 2n	Brown trout 2n
IPN[a]	78		65		87		94			
VHS 1[b]		7		6		68		69	6	2
VHS 3[b]		4		5		90		93	10	56
Non-infected controls	12	1	17	4	11	0	17	0	10	4

[a] For IPNV infections groups of 200 fish were exposed to the virus at an age of 590 degree × days
[b] For VHSV 1 and 3 infections: groups of 100 fish (rainbow trout and hybrids) or 50 fish (brook trout and brown trout) were exposed to the virus at an age of 1550 degree × days

Table 3. Mortality of rainbow trout × brook trout (RT × BT) 3n hybrids and their rainbow trout 2n or 3n half-sibs, and in some experiments rainbow trout × coho salmon (RT × CS) following exposures to the rhabdoviruses VHSV 1 (07/71 and 34/86), VHSV 3 (23/75) or IHNV (32/87). Values in parentheses represent % mortality in non-infected controls

Year	Fish age (degree × days)	No. fish group^{-1}	Virus strain	% Mortality RT × BT	RT × CS	RT
1985	1550	100	07/71	7 (1)	6 (4)	68 (0)
	1550	100	23/75	4 (1)	5 (4)	90 (0)
1986	1200	70	07/71	27 (3)		90 (0)
	1200	70	34/86	18 (3)		100 (0)
	1200	70	23/75	33 (3)		98 (0)
	1700	100	07/71	1 (0)		91 (0)
1987	740	50	07/71	6 (1)		74 (1)
	1200	100	07/71	4 (1)		80 (0)
	1380	100	07/71	4 (0)		72 (1)
	2100	50	07/71	0 (0)		78 (0)
1988	1150	30	07/71	3 (0)	6 (0)	44 (0)
	500	30	07/71	60 (14)	36 (18)	53 (3)
	800	30	07/71	12 (0)	22 (3)	70 (0)
	1150	30	07/71	18 (3)	10 (0)	60 (6)
	1950	30	07/71	3 (0)		78 (0)
1990	1450	100	07/71	34 (0)		88 (0)
		100	32/87	7 (0)		80 (0)
1991	1500	100	07/71	9 (0)		71 (0)
		100	32/87	3 (0)		80 (0)
Mean ± SD		All experiments (19)		13.3 ± 15 (1.6 ± 3.1)		77.1 ± 14.1 (0.6 ± 1.5)
		Experiments with RT × CS (6)		17.3 ± 19.7 (3.3 ± 4.9)	14.1 ± 11 (4.8 ± 6.1)	64.2 ± 14.5 (1.5 ± 2.3)

mortality in hybrids. In only one case (1988), with the youngest fish challenged (500 degree × days post hatch), the mortalities of hybrids (60 %) was comparable to the mortalities of rainbow trout (53 %). When RT × CS hybrids were compared with RT × BT, their response to VHSV challenge was similar. In 1990 and 1991, IHNV was used as a challenge virus and the resistance of RT × BT hybrids was apparent, with only 7 and 3 % mortality compared to 80 % among rainbow trout.

Mean mortality among RT × BT hybrids for all experiments was 13.3 %, and among control 2n or 3n rainbow trout it was 77.1 %. In experiments including RT × BT, RT × CS and rainbow trout simultaneously, their respective mortalities were 17.3, 14.1 and 64.2 %.

No VHSV virus was detected in 5 individual brook

trout examined 3 wk and 4 mo post infection with strain 07/71, or in 5 fish infected with 23/75.

There was no recovery of VHSV strain 07/71 or 23/75 from 15 RT × BT hybrids from 3 wk to 5 mo post infection. In contrast, the virus was readily isolated from 5 hybrids with clinical signs of VHS.

Arctic char and rainbow trout × arctic char

The survival of rainbow trout × arctic char (RT × AC) hybrids (40 %) was similar to the 42 % obtained in RT × BT hybrids (Table 4). Survival of diploid rainbow trout controls was 84 %. The hatching of RT × AC hybrids followed the same pattern as the RT × BT hybrid. When the RT × AC hybrids were challenged with IPNV a high mortality occurred (76 %) that correlated with a similar mortality (61 %) in arctic char in a parallel challenge (Table 5). The highest mortality (85 %) was recorded in rainbow trout. IPNV was isolated from all moribund arctic char or hybrids checked. The mortality rate of arctic char exposed to $VHSV_1$ did not exceed the mortality recorded in non-exposed controls. This was also true for $VHSV_3$, but in that case the young arctic char were still undergoing unexplained mortalities (39 % during the course of the experiment in non-infected controls). The RT × AC hybrid displayed a low but significant mortality when infected with $VHSV_1$ (31 and 18 % mortality in the 2 trials). Mortalities among rainbow trout challenged in parallel were 90 and 91 % respectively. The mortality caused by $VHSV_3$ reached 43 % in hybrids and 96 % in rainbow trout. The virus was re-isolated from all moribund fish.

Table 4. Survival to first feeding of rainbow trout × brook trout (RT × BT) 3n hybrids compared to rainbow trout × arctic char (RT × AC) 3n hybrids. The ova of each of 5 rainbow trout females were equally distributed into the 4 groups. The ova were fertilized with milt from 10 sires of the respective species

	RT × BT 3n hybrids	RT × AC 3n hybrids	RT 2n
Total no. of eggs	1757	1547	2581
No. of fry at start of feeding	738	623	2156
Survival (%)	42	40	84
Survival (% of 2n RT)	50	48	100

Lake trout and rainbow trout × lake trout hybrids

No fish survived beyond the stage of yolk sac resorption in the conventional cross of rainbow trout females with lake trout (LT) males (Table 6). When a heat shock was applied, 39 % of the offspring survived. Hatching of RT × LT hybrids occurred ca 4 d later than that of rainbow trout. When young lake trout were exposed to IPNV, only 16 and 26 % of the fish died compared to 94 and 56 % in rainbow trout controls (Table 7). IPNV was re-isolated from all of the dead lake trout tested (n = 10), and from 3 survivors 2 mo later. Lake trout were also found to be susceptible to $VHSV_1$ and $VHSV_3$, with a mortality of 47 and 30 % respectively compared to 88 and 98 % in rainbow trout controls. Moribund lake trout displayed clinical signs of VHS, including exophthalmia and haemorrhages, and the virus was easily recovered from dead fish. The RT × LT hybrids displayed intermediate susceptibility to IPNV (60 % mortality compared to 85 % in rainbow trout), with the clinical signs of the disease (corkscrew swimming and swollen abdomen). The RT × LT hybrids experienced a 20 % mortality following exposure to VHSV.

Table 5. Final mortality (%) in groups of rainbow trout × arctic char (RT × AC) and rainbow trout × brook trout (RT × BT) 3n hybrids, and in 2n rainbow trout and arctic char following infection with IPNV and VHSV serotype 1 and 3

Virus	RT × AC 3n hybrids		RT × BT 3n hybrids	AC 2n		RT 2n	
IPN[a]	76			61		85	
VHS 1[b]	31	18	1	6		90	91
VHS 3[b]	43				38		96
Non-infected controls	7	0	0	11	39	0	0

[a] For IPNV infections groups of 100 fish were exposed to the virus at an age of 560 degree × days (RT × AC 3n hybrids and RT 2n) and 750 degree × days (AC 2n)

[b] For VHSV infections groups of 100 fish were exposed to the virus at an age of 1700 degree × days (RT × AC and RT × BT 3n hybrids and RT 2n) and 750 degree × days (AC). Two different groups of arctic char of the same age were used for infection with VHS 1 and 3

Table 6. Survival to first feeding of rainbow trout × lake trout (RT × LT) 2n and 3n hybrids compared to 2n and 3n rainbow trout. The ova from each of 5 rainbow trout females were equally distributed into the 4 groups. The ova were fertilized with milt from 12 sires of the respective species

	RT × LT 2n	RT × LT 3n	RT 2n	RT 3n
Total no. of eggs	327	531	349	467
No. of fry at start of feeding	0	208	280	370
Survival (%)	0	39	80	79
Survival (% of 2n RT)	0	48	100	98

DISCUSSION

Following heat shock, triploid hybrids were obtained between rainbow trout (now placed in the genus *Oncorhynchus*) and 3 species of char (genus *Salvelinus*) used as sires. The best survival rate obtained for RT × BT triploid hybrids (62% of the diploid control) is comparable to the 66% obtained by Chevassus et al. (1983), and approximates the yield of parallel crosses between rainbow trout and coho salmon. We obtained a survival rate of 48% for RT × AC triploid hybrids similar to that reported by Blanc & Poisson (1988), and equivalent to that obtained with RT × BT. With lake trout used as sires, we confirmed that no diploid hybrid survived until the end of resorption, a situation previously reported by Buss & Wright (1956), Seguin (1957) and Sutterlin et al. (1977). The survival rate of the RT × LT hybrids obtained by heat shock (48% of the rainbow trout diploid control) fell within the range of the 2 other hybrids.

Susceptibility of RT × BT to IPNV was not surprising, since both parental species are highly susceptible to this virus. In contrast, both the hybrid and the male parental species (BT) proved to be almost totally resistant to VHSV 1 and 3. The resistance of brook trout in our study contrasts with an earlier report of Rasmussen (1965), who obtained mortality following infections with VHSV However, his experiment lacked non-infected controls and was performed at a temperature which reached 20°C, suggesting a bacterial disease could have been responsible for the mortalities observed.

The resistance of RT × BT was confirmed in 12 cases out of 13 for VHS_1 (strain 07-71). In one trial in 1988, the mortality of hybrids was not statistically different from that of the rainbow trout control. That trial used the youngest fish ever tested. As previously observed (Dorson & Chevassus 1985, unpubl. results), there were no differences between the susceptibility of diploid and triploid rainbow trout to IPNV or VHSV. This finding allowed the use of either as positive controls in trials with the hybrids. The resistance of RT × BT hybrid against the 2 IHNV challenges is promising and needs confirmation with other IHNV strains.

Arctic char were found to be susceptible to IPNV but less so than rainbow trout. As expected the RT × AC hybrids experienced a mortality intermediate between the 2 parental species. No mortality could be attributed to $VHSV_1$ or to $VHSV_3$ in arctic char. The resistance of the RT × AC hybrids against these 2 isolates was obvious, but lower than the resistance of their RT × BT half-sibs in the same experiment.

Some resistance of lake trout to IPNV was expected, since Silim et al. (1982) have shown that this species was far less susceptible to IPNV (3 different isolates from Canada, considered as belonging to VR-299 serotype) than rainbow trout and brook trout. We have confirmed here the almost complete resistance of lake trout to a pathogenic strain of IPNV serotype Sp. Unfortunately, as with coho salmon (Dorson & Chevassus 1985), this resistance was not sufficiently transmitted to the offspring. The lake trout were unique among the 3

Table 7. Final mortality (%) in groups of lake trout and rainbow trout × lake trout (RT × LT) 3n hybrids infected with IPNV and VHSV 1 and 3

Virus	RT × LT 3n hybrids		RT 2n		LT 2n			RT 2n		
IPN[a]	60		85		16	26		94	56	
VHS 1[b]		20		82			47			88
VHS 3[b]							30			98
Non-infected controls	16	0	8	0	6	14	1	0	3	0

[a] For IPN infections of hybrids and rainbow trout control, groups of 250 fish were exposed to the virus at an age of 350 degree × days. For IPN infections of lake trout and rainbow trout control, 2 different experiments were performed with a 1 yr interval: groups of 100 fish were exposed to the virus at an age of 550 degree × days (first year), or 350 degree × days (second year)

[b] For VHSV infections, groups of 100 hybrids and rainbow trout control were exposed to the virus at an age of 1100 degree × days, and groups of 70 lake trout and rainbow trout control were exposed to the virus at an age of 1650 degree × days

Salvelinus spp. tested regarding their undeniable susceptibility to VHSV. The mortality among lake trout was higher with $VHSV_1$ than with $VHSV_3$ ($VHSV_3$ is usually more pathogenic for rainbow trout than $VHSV_1$). The RT × LT hybrids were susceptible to $VHSV_1$, although their mortality was lower than in both parental species. Because of the high susceptibility of the lake trout to VHSV, further experiments were not carried out with this species.

Conclusions regarding the susceptibility or resistance of fishes must consider both their genetic variability as well as the diversity of the viruses. Although we have used representative strains of the 3 most important viruses associated with natural outbreaks of disease, these strains represent a very small sample of the total number available. It is therefore conceivable that a strain of VHSV pathogenic for brook trout will be isolated in a manner similar to the relatively recent discovery of VHSV type 3 which is pathogenic for brown trout. A report exists to show that VHSV isolates belonging to serotype 1 can also be pathogenic for brown trout (Schlotfeldt & Ahne 1988).

A virus can also become adapted to a new species. A disquieting example has been published by Hedrick et al. (1987) in the case of IHNV: hybrids between coho salmon (historically considered as refractory) and chinook salmon (susceptible) are susceptible to the disease, and the hybrids may have served as a suitable host for adaptation of IHNV to coho salmon, as indicated by individual coho suffering from IHN.

Another limitation of our study was the use of populations of fish which could (with the exception of the rainbow trout 'synthetic' strain) represent a very small part of the genetic composition of the species. This might give an incomplete picture of the susceptibility or resistance of the host to a given pathogen.

Great differences in the susceptibility to certain disease agents have been found among different populations of the same species of fish (reviewed by Chevassus & Dorson 1990). This variability would also be expected to affect hybrids.

With these reservations in mind, it is apparent that the RT × BT hybrids represent the most promising candidate, at least in France, for a hybrid that resists diseases caused by rhabdoviruses. The RT × BT hybrids provide the same advantages and disadvantages as RT × CS hybrids, including resistance to IHNV and susceptibility to IPNV (even though the mortality due to IPNV was statistically lower in RT × CS than in rainbow trout controls). Coho salmon is presently not included in autochthonous or acclimatized species in France and its importation is more or less restricted. In contrast, brook trout are reared in numerous farms, and are officially considered as belonging to France's fauna. The growth of RT × BT hybrids has been found to be intermediate between the 2 parental species, yet not substantially different from rainbow trout during the first year (Quillet et al. 1988a). However in some cases the growth of hybrids exceeded that of normal rainbow trout after 2 yr due to the sterility of hybrids. This makes hybrids particularly suitable when large fish are sought. It is also obvious that the mortality which affects the hybrid in its young stages (eggs mainly) is more easily tolerated by the breeder than losses provoked by rhabdoviruses among market-sized fish. One method to further reduce mortalities due to rhabdoviruses in the young hybrids is to keep them on spring water supplies as long as possible. This practice also greatly reduces the risk of IPN.

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