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Comparisons of cultured triploid and diploid Atlantic salmon (*Salmo salar* L.)

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The use of sterile, triploid salmon for aquaculture would be an effective method of preventing genetic interactions between cultured and wild salmon. The studies presented in this paper compare the performance of six year classes of triploid and diploid salmon in freshwater and seawater stages of commercial production. Freshwater growth was comparable between triploids and diploids. Freshwater survival was also similar between triploids and diploids except that survival was lower in the triploids for the developmental interval between fertilization and first feeding. In sea water, triploids performed better than diploids in terms of growth. However, survival was lower in triploids and they showed a higher incidence of jaw deformities. In summary, the overall yields of triploids was lower than diploids under culture conditions.

Key words: deformities, growth, size, survival, triploidy.

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Introduction

Sterilization of cultured Atlantic salmon has two benefits, namely, the prevention of gene flow from aquaculture escapees to wild stocks and the control of sexual maturation in aquaculture. Triploid induction is an effective way of producing sterile fish and is the only sterilization method available on a commercial scale (McGeachy *et al.*, 1994, 1995). If fish farmers are to be readily encouraged to use triploid Atlantic salmon (*Salmo salar* L.), then their performance must equal or be superior to diploid fish under commercial production. However, even if the performance of triploid fish is inferior to diploid fish, the farmers may need to be persuaded to use triploids if the costs of introgression with the wild fish are high. The studies reported in this paper compare the performance of triploids, relative to diploids, for both fresh- and seawater stages of aquaculture production.

Materials and methods

Atlantic salmon from 5 year classes of four domestic strains, developed by the Salmon Genetics Research Program (SGRP), Atlantic Salmon Federation, St Andrews, New Brunswick, Canada, were used to evaluate the performance of triploid Atlantic salmon in the aquaculture industry. Fish from the 5 year classes included; the 1990 and 1994 year classes of Strain 90JC, the 1991 year class of Strain 87JC, the 1992 year class of Strain 84JC, and the 1993 year class of Strain 85XC. The breeding practices used to establish these aquaculture strains and strain designations are described by Friars *et al.* (1995). In addition, two year classes from the SGRP's multiplier stock, 1994 and 1995, were used to evaluate the performance of triploid salmon at a commercial hatchery. Multiplier stock are genetically improved fish from SGRP aquaculture strains.

In 1993 and 1994, all-female triploids were produced in strains 85XC and 90JC respectively by using milt

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Table 1. Freshwater survival, from fertilization to first-feeding, and S1 smolt rate for diploid and triploid Atlantic salmon.

Strain	No. of families	Year class	Survival from fertilization to first feeding (%)		S1 smolt rate (%)	
			Diploid	Triploid	Diploid	Triploid
90JC	10	1990	56.2	42.9	80.8	76.5
87JC	20	1991	63.4	41.0**	76.2	67.0*
84JC	9	1992	66.9	67.8	87.6	86.0
		1992[CH]			85.1	74.0**
85XC[F]	20	1993	51.7	41.9	94.6	95.5
90JC[F]	27	1994	24.2	22.0	92.3	87.7
MG		1994[CH]	77.6	5.6		
MG		1995[CH]	33.3	6.3		

*Significant at $p<0.05$; **significant at $p<0.01$; [CH]commercial hatchery; [F]all-female stocks.

from sex-reversed females to fertilize eggs prior to triploidy induction. Triploids produced in 1990, 1991, 1992, and 1995 were mixed sex (males and females).

Triploids were produced by pressure shock. A laboratory-scale pressure vessel, with the capacity to shock 500 eggs at a time, similar to that described by Benfey *et al.* (1988), was used to produce triploids in 1991 and 1992. A commercial-scale vessel with the capacity to pressurize 5000 eggs at a time was used after 1992. In all cases, triploidy was induced by subjecting the eggs to a pressure of 9500 psi for 5 min, beginning three hundred degree (°C) minutes after fertilization. Triploid induction success was determined by measuring erythrocyte DNA content using a flow cytometer. For each year class, 10 fish from each of the triploid families, together with 10 fish from one diploid family were tested 6 months after fertilization. The diploid blood samples served as a baseline control. All blood samples taken from fish in pressure-treated groups were verified as being triploid. This indicates that the hydrostatic pressure treatment which was used for triploidy induction was fully effective.

Hatchery facilities at the Atlantic Salmon Federation were used to evaluate freshwater performance in five year classes. A commercial hatchery was also used to evaluate the performance of the 1992, 1994, and 1995 year classes. Early freshwater survival from fertilization to first feeding was monitored. Fry were measured and weighed 7 months after fertilization. At 14 months post-fertilization, the fish were sorted into two size groups; those greater than 13 cm in length were identified as potential S1 (yearling) smolts and those less than 13 cm in length as parr (potential S2 smolts). Parr and smolts were measured and weighed and the proportion of S1 smolts was calculated for diploids and triploids.

Triploids and diploids were reared communally in fresh water and in most cases in sea water. The exception was the 1992 year class, where triploids and diploids were reared in separate sea-cages at the experimental farm. Sea-cage facilities at an experimental farm were used to evaluate seawater performance of 1990, 1991, and 1992 year classes of strains 90JC, 87JC, and 84JC respectively. Sea-cages at two commercial facilities, A and B, were also used to evaluate seawater performance of strains 84JC and 87JC, respectively. Survival was monitored from smolt transfer to the time of harvest. Fish size was measured at the post-smolt (4 to 5 months in sea water), pre-harvest (12 to 17 months), and harvest (19 to 22 months) stages. Fish were also examined for deformities at the time of harvest. The proportion of grilse, i.e. fish which reached sexual maturity after one sea winter, was also determined for the 1990, 1991, and 1992 year classes.

The number of families in each year class of each strain is given in Table 1. Family size was aproximately 500 individuals in the 1991 and 1992 year classes and 2000 individuals in the 1993, 1994, and 1995 year classes. Sample size was approximately 20 to 30 individuals per family for freshwater and seawater size variables.

Analyses of variances were carried out to determine significant differences between triploids and diploids where family information was available. Where family information was not available, t-tests were used. No statistical analysis was carried out on the percentage data for the incidence of deformities and survival in sea water.

Results

Freshwater performance

Freshwater survival was similar between triploids and diploids except for the developmental interval between fertilization and first-feeding. For most year classes, survival was lower in the triploids at this stage (Table 1).

Diploids and triploids of the 1993 and 1994 year classes reared at the ASF hatchery were all-female. Early

Table 2. Fry, parr, smolt size, and condition factor for diploid and triploid Atlantic salmon.

Strain	Year class	Variable	Fry		Parr		Smolt	
			Diploid	Triploid	Diploid	Triploid	Diploid	Triploid
90JC	1990	Length (cm)	7.7	7.9	11.9	11.9	18.9	18.8
		Weight (g)	5.2	5.5	16.9	16.0	73.3	72.0
		Condition factor	1.11	1.07**	0.98	0.92**	1.06	1.02*
87JC	1991	Length (cm)	7.2	7.5	11.7	11.8	17.0	17.1
		Weight (g)	4.3	4.7	16.4	15.9	52.3	52.9
		Condition factor	1.12	1.09**	1.00	0.96**	1.05	1.02**
84JC	1992	Length (cm)	6.5	6.2**	11.9	12.1	18.0	17.9
		Weight (g)	3.0	2.4**	17.5	17.4	64.1	62.8
		Condition factor	1.05	1.01*	1.02	0.97**	1.08	1.06*
	1992^{CH}	Length (cm)			11.4	12.1**	19.6	18.9**
		Weight (g)			16.2	17.9**	87.2	76.9*
		Condition factor			1.04	0.97	1.13	1.12
85XC^{F}	1993	Length (cm)			10.8	11.3	16.7	17.0
		Weight (g)	3.9	3.3**	12.2	14.2	51.5	55.3
		Condition factor			0.93	0.96	1.08	1.11
90JC^{F}	1994	Length (cm)	6.9	6.7	11.6	12.1**	18.8	18.7
		Weight (g)	3.9	3.4*	16.0	16.5	70.3	68.6
		Condition factor	1.17	1.13	1.01	0.92**	1.05	1.02**

*Significant at $p<0.05$; **significant at $p<0.01$; ^{CH}commercial hatchery; ^{F}all-female stocks.

Table 3. Post-smolt, pre-harvest size, and condition factor for diploid and triploid Atlantic salmon.

Strain	Year class	Variable	Post-smolt		Pre-harvest	
			Diploid	Triploid	Diploid	Triploid
90JC	1990^{EX}	Length (cm)	41.0	42.4*	66.3	68.1*
		Weight (g)	0.83	0.87*	3.46	3.62
		Condition factor	1.16	1.11**	1.16	1.11*
84JC	1992^{EX}	Length (cm)	35.1	36.3*	68.3	67.9
		Weight (g)	0.54	0.57	3.75	3.58**
		Condition factor	1.23	1.17**	1.16	1.13**
	1992^{CB}	Length (cm)	42.6	42.5		
		Weight (g)	0.90	0.86**		
		Condition factor	1.15	1.12**		

*Significant at $p<0.05$; **significant at $p<0.01$; ^{CB}fish size at commercial cage site B; ^{EX}fish size at experimental farm.

freshwater survival for the 1993 year class was similar to previous year classes for both triploids and diploids. Survival for the 1994 year class was low in both triploids and diploids, probably as a result of poor egg and milt quality. Triploid survival was considerably lower at the commercial hatchery than at the ASF hatchery (Table 1). The high mortality at the commercial hatchery peaked between the eyed egg stage and first feeding, resulting in very low survival rates (5.6% and 6.3% for 1994 and 1995 year classes respectively) for the triploids (Table 1). Water quality was tested and fish were sampled for diagnostic purposes. However, no explanation was found for the high mortality. Survival from first-feeding to smolt transfer was similar for triploids and diploids with negligible mortality in both groups.

Freshwater growth was measured at the fry stage and when the parr and smolts were separated. Triploid fry were larger than diploid fry in the 1990 and 1991 year classes, but significantly smaller than diploid fry in the 1992, 1993, and 1994 year classes (Table 2). In three of the year classes, differences between triploid and diploid parr (length and weight) were not significant (Table 2). However, for the 1992 (Commerical hatchery) and 1994 (ASF hatchery) year classes triploid parr were significantly longer than diploid parr (Table 2). In most cases, diploid smolts were longer and heavier than triploid smolts (Table 2). Condition factor was consistently lower for triploid fry, parr, and smolts than for diploid fish (Table 2). The proportion of S1 smolts was found to be significantly higher in the diploid fish of the 1991

Table 4. Harvest size, weight loss, and condition factor for diploid and triploid Atlantic salmon.

Strain	Year class	Variable	Diploid	Triploid
90JC	1990[EX]	Total weight (kg)	2.99	3.20
		Gutted weight (kg)	2.83	3.02
		Weight loss (%)	5.5	5.4
87JC	1991[EX]	Total weight (kg)	3.72	3.79
		Gutted weight (kg)	3.41	3.48
		Weight loss (%)	8.3	8.2
	1991[CA]	Length (cm)	75.5	76.3*
84JC	1992[EX]	Total weight (kg)	4.13	4.51**
		Gutted weight (kg)	3.76	4.16**
		Weight loss (%)	8.7	7.5**
	1992[CB]	Length (cm)	72.3	71.7
		Total weight (kg)	4.36	3.91*
		Gutted weight (kg)	3.91	3.60*
		Weight loss (%)	8.0	7.8
		Condition factor	1.12	1.05*

*Significant at $p<0.05$; **significant at $p<0.01$; [CA]fish size at commercial farm A; [CB]fish size at commercial farm B; [EX]fish size at experimental farm.

(ASF hatchery) and 1992 (Commercial hatchery) year classes (Table 1).

Seawater performance

Seawater growth was measured at the post-smolt, pre-harvest, and harvest stages. Post-smolt measurements indicated that triploid fish were significantly longer and heavier in the 1990 year class (Table 3). Similarly, triploid post-smolts in the 1992 year class reared at the experimental farm were significantly longer. However, triploid post-smolts reared at commercial farm B weighed significantly less than the diploid fish (Table 3). At the pre-harvest stage, triploids were significantly longer than diploids in the 1990 year class (Table 3). Triploid and diploid fish of the 1992 year class were of similar length but diploids were significantly heavier than triploids (Table 3).

Triploids weighed more than diploids at harvest in all year classes except the 1992 year class at commercial farm B (Table 4). Triploids of the 1991 year class reared at commercial farm A were significantly longer than diploids at harvest (Table 4). In addition, total and gutted weight were significantly higher in triploids of the 1992 year class than in diploids at the experimental farm. Percent weight loss from gutting was consistently higher in diploids (Table 4). As in fresh water, condition factor in sea water was consistently lower in triploids than in diploids (Tables 3 and 4).

Jaw and body (scoliosis and lordosis) deformities were observed in both diploids and triploids in fresh water, but became more evident as the fish grew in sea water. By harvest time, in all strains monitored in sea water, the incidence of deformities was higher in triploids than in diploids (Fig. 1).

Diploid and triploid salmon at commercial farm B suffered extremely high mortality due to heavy sea lice infestations (Fig. 2). Diploid fish had higher survival in sea water than triploid fish in other year classes (Fig. 2).

Discussion

Freshwater survival was similar between triploids and diploids, except for the developmental interval between fertilization and first-feeding. Survival was lower in the triploids at this stage probably as a result of the triploidy induction. Similar survival rates have been described for triploid Atlantic salmon produced by hydrostatic shock (Johnstone *et al.*, 1991). The lower survival at such an early developmental stage before first feeding would

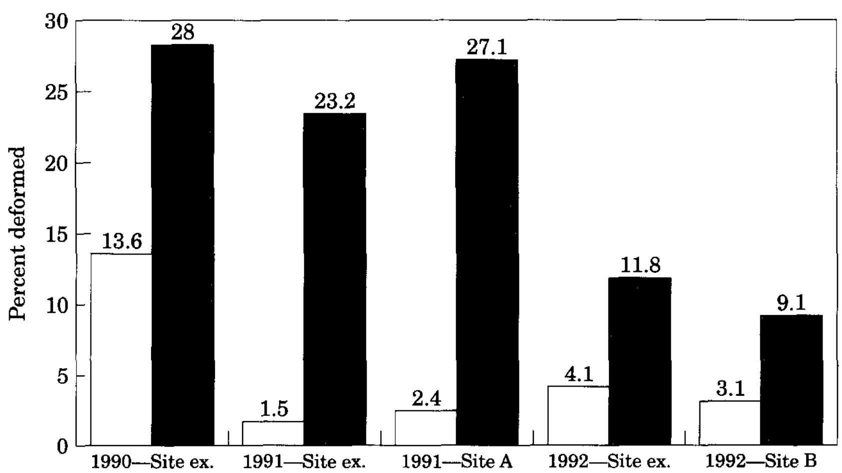

Figure 1. Percentage occurrence of deformities at harvest in three year classes of diploid and triploid Atlantic salmon. Site ex. – experimental farm; site A – commercial farm A; site B – commercial farm B. diploid (□); triploid (■).

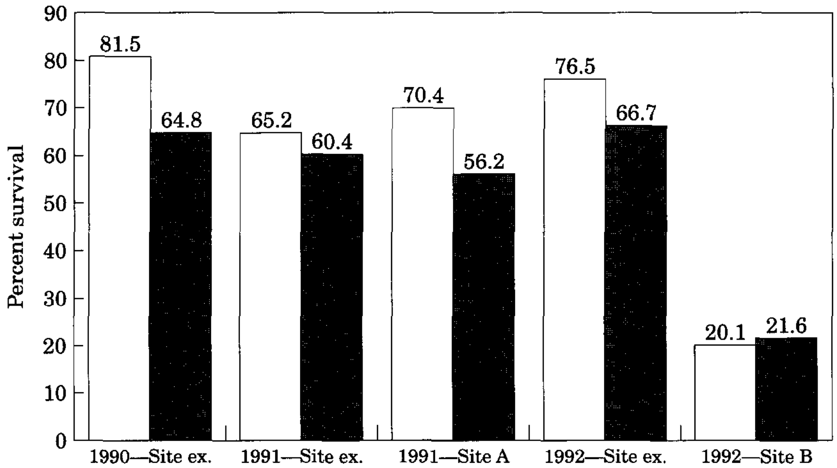

Figure 2. Percentage survival from smolt transfer to harvest in three year classes of diploid and triploid Atlantic salmon. Site ex. – experimental farm; site A – commercial farm A; site B – commercial farm B. diploid (□); triploid (▩).

cause minimal economic losses in the freshwater stage of production.

Triploid fry were significantly smaller than diploid fry in three year classes (Table 2). Similar results have been reported for other studies with the slower growth in triploid fish being attributed to a delay in first-feeding (Jungalwalla, 1991; McGeachy *et al.*, 1995).

The growth of triploid parr and smolts varies between strains/year classes and rearing facilities. However, there is a tendency for significant size differences between triploids and diploids to decrease as the fish grow even though stocking densities and biomass were similar for diploid and triploid groups. This trend has been found in other studies, implying that triploids may have a faster growth rate than diploids. McGeachy *et al.* (1995) found that triploids have a higher specific growth rate than diploids during early freshwater rearing.

With regard to seawater growth, triploids were larger than diploids. Additionally, it is interesting to note that the pre-harvest and harvest size differences between triploids and diploids were more significant when the ploidy groups were reared separately at the experimental farm in 1992. Triploid and diploid groups from all other year classes were reared communally in sea water. Triploids performed better when reared separately and not communally with diploids. The triploids were significantly heavier than the diploids for the 1992 year class and this may be attributed to the absence of diploids. A competition experiment carried out by McGeachy *et al.* (1995) showed that increasing the proportion of diploids when diploids and triploids were reared communally had a negative effect on early freshwater growth of the triploids. This effect was attributed to diploids being more aggressive than triploids and thus more competitive for space and food.

In both fresh water and sea water, condition factor was consistently lower in triploids than in diploids. Other studies have reported that triploids have a lower condition factor, causing then to resemble wild salmon (Johnstone *et al.*, 1991). This may prove advantageous to the marketing of triploids.

In commercial salmon farming, Atlantic salmon mature after either one or two sea winters. Early maturation after one sea winter (grilsing) can reduce the value of the fish at market because of a reduction in flesh quality. Maturation in harvest size fish was monitored in the 1990, 1991, and 1992 year classes. The rate of early maturation was less than 3% in both diploids and triploids in all year classes. This low level should not be a concern to salmon farmers. However, in warmer winters higher grilse rates may occur. No female triploids were found to mature and this may be an advantage of using all-female triploid Atlantic salmon populations in aquaculture.

In this study, the incidence of deformities was higher in triploids than diploids. Jaw deformities were the most prominent and have been described in other studies on farmed triploid Atlantic salmon (Sutterlin *et al.*, 1987; Jungalwalla, 1991; McGeachy *et al.*, 1996). Lower jaw deformities may be due to nutritional deficiencies such as vitamin C (Hughes, 1992). Some studies have indicated that jaw deformities may be related to the fast growth rate of triploids (Lee and King, 1994). In this study the jaw deformities did not appear to affect seawater growth. However, the abnormality does reduce the market value of the fish.

Diploid fish had a higher survival in sea water than triploid fish. Some studies have shown that the survival of triploids is similar to diploids when exposed to acute stress (Biron and Benfey, 1994). Other studies have shown that triploids have lower survival than diploids when exposed to chronic stress (Ojolick *et al.*, 1995). The reduced survival in triploids may be attributed to the lower haemoglobin-oxygen loading ratios found in triploids resulting in a reduced oxygen-carrying capacity (Benfey, 1991), requiring triploids to increase their cardiac output during periods of stress (King and Lee, 1993). This may explain the lower seawater survival in triploids in this and other studies where triploids did not survive as well as diploids under unfavourable conditions (Johnson *et al.*, 1986; Quillet and Gaigon, 1990; Benfey, 1991; Jungalwalla, 1991).

The results from this study indicate that triploid Atlantic salmon perform similarly to diploids during the freshwater stage of commercial production and better than diploids in terms of seawater growth. However, yields of triploids are lower because of the decreased seawater survival. In addition, the presence of deformities results in a lower unit price. The lower seawater survival and higher incidence of deformities than in diploids are the major concerns that need to be investigated in triploids. In this study, the seawater evaluation was carried out on mixed-sex stocks reared, for the most part, communally. Future studies should involve the commercial rearing of all-female diploids and triploids in separate cages.

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