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Triploidy induced by pressure shock in Arctic charr (*Salvelinus alpinus*): growth, survival and maturation until the third year

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Abstract − Retention of the second polar body for the production of triploid Arctic charr (*Salvelinus alpinus*) was induced by 65 MPa (650 bar) pressure shocks applied 30, 40 or 50 min after fertilization, each shock lasting 5 min. Triploid yield (percentage of relative survival to control × percent triploidy) varied from 55 to 100 % and it was generally superior to 80. Despite increased mortality and decreased growth in diploid charrs during the spawning period, growth and survival did not differ between diploid and triploid Arctic charr up to and during their third year of life. Most of the triploid males had developed testis, but they did not render milt; few triploid females had developed ovaries. Fecundity of triploid females was low and fertilized eggs from triploid females did not hatch. © 2001 Ifremer/CNRS/Inra/IRD/Cemagref/Éditions scientifiques et médicales Elsevier SAS

fecundity / mortality / pressure shock / reproduction / triploidy

Résumé − Triploïdie induite par choc hyperbare chez l'omble chevalier (*Salvelinus alpinus*) : croissance, survie et indice de maturation jusqu'à l'âge de 3 ans. La production d'ombles chevaliers (*Salvelinus alpinus*) triploïdes par rétention du second globule polaire est réalisée par un choc hyperbare à 65 MPa (650 bar), d'une durée de 5 min appliqué durant 30, 40 ou 50 min après la fécondation. Le rendement de triploïdie (pourcentage de survie relative par rapport aux témoins × pourcentage de triploïdes) varie de 55 à 100 % et il est généralement supérieur à 80. La croissance et la survie des ombles chevaliers triploïdes ne diffèrent pas de celles des ombles diploïdes jusqu'à la fin de la troisième année de la vie des poissons, bien que les diploïdes présentent une mortalité accrue et une croissance ralentie au cours de la période de reproduction. À l'âge de trois ans, la plupart des mâles triploïdes possèdent des testicules développés, mais ils ne produisent pas de laitance. Quelques femelles triploïdes développent des grappes ovariennes. La fécondité des femelles triploïdes est faible et leurs ovules fécondés ne parviennent pas à éclore. © 2001 Ifremer/CNRS/Inra/IRD/Cemagref/Éditions scientifiques et médicales Elsevier SAS

fécondité / mortalité / choc hyperbare / reproduction / triploïdie

1. INTRODUCTION

During the 1990s, interest in the culture of Arctic charr (*Salvelinus alpinus*) increased in France and in northern countries, both for restocking and consumption. By 1999, the production of sterile triploid females of rainbow trout (*Oncorhynchus mykiss*) for the fresh and smoked fish markets had reached 10 000 t in France (Haffray et al., 1999). Sterile triploid Arctic charr could be of similar interest for aquaculture if quality traits (pigmentation, lipid content in the muscle, gutted yield, etc.) and/or rearing characteristics (survival, growth, feed conversion index) could be enhanced relative to mature diploids.

Triploidy induction is well documented in salmonids and many other fish (Ihssen et al., 1990). Spontaneous triploidy was reported in a hatchery population of brook trout *Salvelinus fontinalis*, a species closely related to Arctic charr (Allen and Stanley, 1978). Initial experiments to induce triploidy in this

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species involved treatments with colchicine or cold shock (Smith and Lemoine, 1979; Lemoine and Smith, 1980). Heat shock was also used to produce allotriploids (Scheerer and Thorgaard, 1983) or autotriploids (Dube et al., 1991) in the same species. But most of the heat shock treatments that were efficient in achieving a triploid rate near 100 % had a deleterious effect on eggs. Nevertheless, Galbreath and Samples (2000) obtained a triploid yield of 70 % by the optimization of the thermal shock protocol in brook trout. But the same authors indicated that a difference of one degree in the thermal shock temperature widely changed survival (+1 °C) or triploid rate (–1 °C). Arai (1984) was the first to use hydrostatic pressure on charrs (the Japanese charr *Salvelinus leucomaenis*) at 650 bar for 7 min, 15 min after fertilization, but the treatment induced abnormal embryogenesis and mosaicism with either hyperdiploidy or hypoploidy. Subsequent trials at 500 (Boulanger, 1991) or 650 bars (Deeley and Benfey, 1995) were applied for 5 min at 20 min after fertilization for triploidy induction in brook trout, but these studies provided no information on hatching, survival and the number of progeny analysed for ploidy evaluation. Similarly, in Arctic charr, initial trials of triploidy induction at 650 bar for 5 min applied at 30, 45, 75 and 90 min after fertilization (O'Keefe and Benfey, 1995) also did not provide precise information on the performance and number of progeny analysed for ploidy control. Arctic charr eggs require a lower temperature from fertilization to hatch than do other salmonids (Jungwirth and Winkler, 1984). Consequently the choice of pressure shock rather than thermal shock for the production of triploid could be judicious in this species. Moreover, in rainbow trout, pressure shock seems to give better results than heat shock under experimental conditions as well as in French fish farm trials (Chourrout, 1984; Chourrout et al., 1986; Haffray et al., 1999). In contrast, Palti et al. (1997) obtained a comparable efficiency to prevent extrusion of the second polar body by pressure or thermal shock in the same species.

Growth, reproduction and survival of triploids are well documented in salmonids (Ihssen et al., 1990), but data are limited for *Salvelinus* species, being available for brook trout only. Sterility in spontaneous triploids of brook trout has been associated with a high rate of mosaicism (Allen and Stanley, 1978) and comparative studies suggest that growth of females after the onset of sexual maturation is greater in triploids than diploids (no comparable data are available on diploid and triploid males) (Boulanger, 1991). The proportion of somatic mass was greater in 3-year-old triploid females than in diploids (78 vs. 68 %), the difference being due to greater gonad development in diploids. Triploid brook trout were not found to differ from diploids in learning ability (i.e. to avoid an electric shock using a Y-maze test), haemoglobin level, metabolic rate, swimming performance, feeding behaviour, comparative growth until 250 g (age group 1+) and food consumption (Deeley and Benfey, 1995; Stillwell and Benfey, 1995; O'Keefe and Benfey, 1997, 1999). These trials indicate that rearing of triploid brook trout in tanks of freshwater, at optimal temperature and at medium capacity (12 $kg \cdot m^{-3}$) does not seem to induce a decrease in performance relative to diploids. Evaluation of triploid Arctic charr performance needs to be assessed, at least until the onset of sexual maturation in females, to obtain information on their breeding characteristics.

The aim of the present study was to assess the efficiency of hydrostatic pressure shock to induce triploidy in Arctic charr, to compare the growth of triploid and diploid controls from the onset of exogenous feeding until the end of the third year of life and to determine rates of mortality and sterility in triploid fish during the first reproductive period.

2. MATERIALS AND METHODS

2.1. Source of spawners and fertilization

The experiments were conducted from 1994 to 1997 in rearing facilities of the Institut National de la Recherche Agronomique, located on the shores of Lake Geneva at Thonon-les-Bains, Haute-Savoie, France, using hatchery-reared Arctic charr derived from Lake Geneva stocks. Brood fish were kept in 12-m^3 rearing tanks supplied with water pumped from a depth of 52 m in Lake Geneva. Females weighed 1 kg or more, their fecundity exceeded 3 000 ova. On 21 February 1994, the eggs of four 4-year-old females were fertilized separately with a mixture of sperm from five males according to Gillet (1991). After mixing sperm and eggs, a saline buffered diluent was added (Billard, 1977). Fifteen minutes after fertilization, the eggs were transferred to freshwater for incubation at 8 °C.

2.2. Pressure treatments

In order to induce retention of the second polar body, pressure shocks were applied 30, 40 and 50 min after fertilization with the press described by Foisil and Chourrout (1992). The spawn of the four different females were treated separately. In each treatment, 400 to 500 eggs were placed in a titanium cylinder filled with water at 8 °C and closed by a piston. The pressure level was raised with an electric press and reached 65 MPa (650 bar) after 2 or 3 s. At the end of the treatment, which lasted 5 min, decompression was instantaneous.

2.3. Incubation of eggs

Egg batches from the various pressure treatments and controls were incubated separately at 6.5 °C (i.e. sixteen different egg batches). Dead eggs were counted and removed weekly. Survival rates were calculated for each egg batch at the end of eyed stage. Chi-square tests were used to compare observed and expected

survival rates. Egg batches were incubated separately until swim-up stage but mortality was not recorded after eyeing.

2.4. Ploidy analysis

Ploidy investigations were performed on newly-hatched Arctic charr by flow cytometry using a Partec CA II following the protocol of Lecommandeur et al. (1994). Per female, 24 specimens were analysed in control groups and 54 in each pressure treatment. Triploid yield was calculated as a percentage of relative survival to control × percent triploidy. Pressure treatment groups and controls from the spawn of female 4 were not analysed for ploidy determination.

2.5. Rearing of juveniles

The experimental groups for which triploidy was 100 % were combined at the swim-up stage for subsequent rearing. Diploid control groups were also combined. Diploid and triploid charr were reared in two different 120-L troughs. Fish were fed 12 h daily by automatic feeders using trout commercial dry pellets in excess of satiation. On 3 August 1994, day 0 of the growth trial, 100 fish from each group were anaesthetized with 2-phenoxy ethanol ($0.3\ mL \cdot L^{-1}$) and weighed to the nearest 0.1 g. Thereafter, 500 diploid fish were marked by adipose fin ablation, then combined with 2 500 triploid charrs in the same 2 000-L rearing tank. Water was pumped from Lake Geneva, its temperature was continuously recorded by a probe type Minilog TR (Vemco, Canada). Water temperatures varied each year from 6 °C at the end of winter to 9 °C at the end of summer. Fish were fed at 1 % of body weight. One-hundred diploid and triploid fish were weighed individually on several occasions: 7 October 1994 (day 65), 10 November 1994 (day 99), 21 February 1995 (day 202), 29 June 1995 (day 330) and on 22 March 1996 (day 597). Mortality was not recorded.

2.6. Rearing of spawners

On 26 May 1996, the fish were graded by weight, then 100 diploid and 100 triploid fish (> 125 g) were selected and reared in the same 2 000-L tank for another 443-d growth trial (from 26 May 1996 to 12 August 1997). Mortality was recorded daily. The fish were weighed every 2 months. From the beginning of the spawning period (i.e. from the end of November 1996 until March 1997), 3-year-old fish were regularly examined once a week to detect ovulation and to determine the percentage of mature males, mature females and immature fish. Each ovulated female was anaesthetized, weighed and the eggs collected. Ovarian fluid was removed and the eggs were weighed to the nearest 0.1 g. About fifty ova from each female were weighed to the nearest 1 mg to calculate mean egg weight. Relative fecundity, expressed as the number of $eggs \cdot kg^{-1}$ body weight, was calculated for each female. Relative fecundity in diploid and triploid females was compared using the median test (Sprent, 1989). Eggs produced by three triploid females were fertilized with milt from diploid males as described above. During the spawning period and soon after, charr that died from fungal diseases in both diploid and triploid groups were dissected. Five immature triploids were sacrificed and dissected. The gonado-somatic index (GSI: 100 × weight of gonad/body weight), the visceral-somatic index (VSI: 100 × weight of viscera/total body weight) were calculated for each fish. In eviscerated charrs, the carcass-somatic index, CSI: 100 % – (GSI + VSI) was calculated for males, females and immatures in both diploid and triploid group. Chi-square tests were used to compare mortality rates between diploid and triploid groups. Student's unpaired *t*-tests were used to compare fish weights in diploid and triploid groups.

3. RESULTS

3.1. Egg viability

Survival rates at the eyed stage varied widely between the spawn of different Arctic charr females but were within the range 40–80 % for the control groups. In egg batches from two females, pressure treatments never induced an increase in mortality and in the other two egg batches, a significant increase in mortality (> 15 %) was observed on two occasions only. In ten of twelve pressure treatment groups, relative survival was ≥ 80 % and egg survival did not differ significantly from the control (*table I*).

3.2. Ploidy analysis

Flow cytometric analysis revealed that a pressure treatment applied 40 min after fertilization always produced 100 % triploids. Triploidy was not systematically induced by a pressure shock applied 30 or 50 min after fertilization, although the rate of triploidy was always higher than 85 %. Triploid yield varied from 55 to 100 % and was generally superior to 80 % (*table I*).

3.3. Juvenile growth trial

At the end of the 597-d growth trial, at age 2, the mean weight of diploids was 17 % greater than that of triploids, although this difference was not statistically significant (*figure 1*). Growth rates of both diploids and triploids decreased quickly from day 65 (7 October 1994) to day 202 (21 February 1995). In the meantime, the coefficient of variation for weight increased in both diploid and triploid charrs (*figure 1*).

3.4. Brood stock growth trial

During the months that preceded the first spawning period, at age 3, the weight of diploid fish increased

Table I. Egg survival and triploid yield in Arctic charr after a 5-min application of 65 MPa initiated at 30, 40 or 50 min post fertilization. Eggs of female No. 4 were not analysed for ploidy determination. * $P < 0.05$, compared to control from the same spawn; –: data unavailable.

	Treatment															
	Control				Pressure shock											
					30–35 min				40–45 min				50–55 min			
Female No.	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Diploid number	24	24	24	–	0	2	2	–	0	0	0	–	7	0	0	–
Triploid number	0	0	0	–	54	52	52	–	54	54	54	–	47	54	54	–
Survival at eyed stage (%)	79	66	59	41	75	38*	41*	40	77	60	50	33	74	64	63	43
Relative survival (%)	100	100	100	100	95	58	69	98	97	91	85	80	94	97	106	105
Triploid yield (%)					95	55	67	–	97	91	85	–	82	97	100	–

more rapidly ($P < 0.01$) than that of triploid fish. During the spawning period (days 190–280), the growth of diploids ceased and the weight of triploids became similar to that of diploids. Thereafter, the weight of diploid and triploid fish did not differ significantly. Mortality was higher ($P < 0.01$) in diploids than in triploids during the spawning period. However, 443 d after the beginning of the experiment, cumulative mortality was very similar in diploid and triploid Arctic charr (*figure 2*).

At age 3, 94 % of the diploids were mature. Among the triploid Arctic charr, mature males and mature females represented 51 and 13 %, respectively (i.e. a total of 64 % mature triploids, *table II*).

Percentages of mature females among triploid and diploid Arctic charr were statistically different ($P < 0.01$). Fecundity and gonado-somatic index for

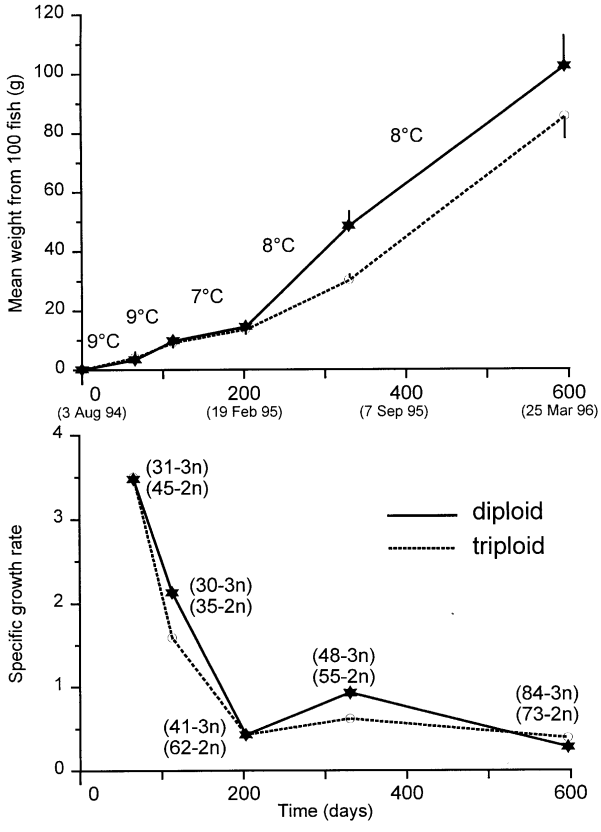

Figure 1. Growth and specific growth rate of diploid (solid lines) and triploid (dotted lines) Arctic charr from August 1994 to March 1996. Mean water temperatures between two consecutive weighing are indicated above the curves. Values in brackets represent the coefficient of variation of weights. Vertical bars represent standard error.

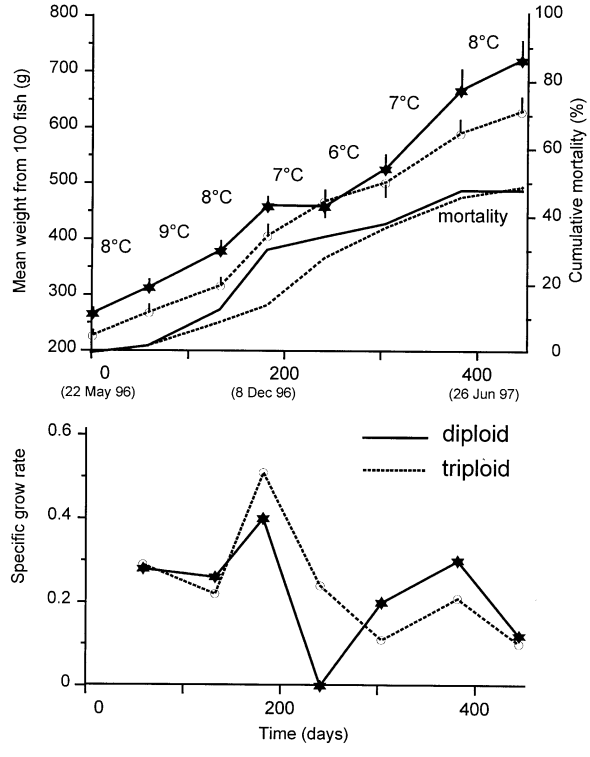

Figure 2. Growth, mortality and specific growth rate of diploid (solid lines) and triploid (dotted lines) Arctic charr from May 1996 to August 1997. Mean water temperatures between two consecutive weighing are indicated above the curves. Vertical bars represent standard error.

Table II. Body mean weight, percentage of mature and immature fish, GSI, VSI, CSI, egg mean weight and relative fecundity in diploid and triploid Arctic charr in winter 1996/97. Fish were 3 years old. Body mean weight, percentage of mature and immature fish were calculated when charrs were weighed on 20 January 1997. –: Data unavailable.

Ploidy	Maturity	Mean body weight (g)	Occurrence (%)	Gonado somatic index (%)	Mean egg weight (mg)	Relative fecundity (egg·kg^{-1})	Viscero somatic index (%)	Carcass somatic index (%)
Diploids	Mature females	488 ± 27	56	16.2 ± 0.7	45.5 ± 1.3	3 617 ± 295	7.8 ± 0.3	76.0 ± 0.9
	Mature males	417 ± 17	38	4.8 ± 0.6			7.9 ± 0.7	87.3 ± 0.9
	Immature (males + females)	443 ± 75	6	–			–	–
Triploids	Mature females	559 ± 85	13	5.4 ± 2.3	58.4 ± 0.3	1 236 ± 370	–	–
	Mature males	417 ± 18	51	3.3 ± 0.7			6.4 ± 1.3	90.3 ± 1.2
	Immature (males + females)	505 ± 26	36	1.1 ± 0.3			8.3 ± 1.1	90.6 ± 1.0

the four triploid females that matured were significantly lower than in diploids (*table II*). On three occasions, ova from triploid females were fertilized with milt from diploid males. All the eggs died before hatching.

Percentages of mature males among triploid and diploid Arctic charr were not statistically different. In diploid males, testes were white-coloured whereas in triploids, testes were pink-coloured. Milt could not be manually stripped from the triploid males, despite being mature.

Carcass somatic index (CSI) did not differ between immature triploids, diploid and triploid males of Arctic charr. CSI of diploid females were significantly inferior to that of immature triploids (*table II*).

4. DISCUSSION

4.1. Induction of triploidy

Survival rates at the eyed stage in the present experiment corroborated previous reports of egg viability in Arctic charr brood stock (Gillet, 1991). In controls, egg survival varied widely between the spawn of different female Arctic charr, corroborating the results of De March (1995) who observed that different spawns from the same group of Arctic charr brood stock often exhibit widely differing survival rates. In Arctic charr, egg survival was similarly high and did not differ from the controls for most of the pressure treatments at 65 MPa applied between 30 and 50 min after fertilization, corroborating previous reports for experimentally-produced triploids in rainbow trout (Lou and Purdom, 1984; Chourrout, 1984) in landlocked Atlantic salmon (Benfey and Sutterlin, 1984a) and in coho salmon (Teskeredzic et al., 1993). Benfey and Sutterlin (1984a) reported that pressure shocks of longer duration (9 to 15 min) or higher magnitude (7.9 to 10.5 MPa) resulted in a drastic increase of mortality in Atlantic salmon. Further studies will be required to test the effects of pressure treatment intensity on egg viability in Arctic charr. Moreover egg mortality, in the present experiment, was only registered from fertilization to eyed stage, so we cannot exclude that cumulative mortality from eyed stage to hatching increased more rapidly in the pressure treatment groups than in control, as observed in rainbow trout (Lou and Purdom, 1984).

The 40-min pressure treatment produced 100 % triploid Arctic charr with excellent survival, relative to the control groups, corroborating works elsewhere for the same species (O'Keefe and Benfey, 1995) and on other salmonids (Lou and Purdom, 1984; Chourrout, 1984; Benfey and Sutterlin, 1984a, 1984b; Teskeredzic et al., 1993). Optimization of the efficiency of thermal and pressure shocks to induce retention of the second polar body required large-scale investigations on rainbow trout (Palti et al., 1997) and for the optimization of thermal shock in brook trout (Galbreath and Samples, 2000). It will be necessary to undertake new experiments in order to optimize the protocol for induction of triploidy in Arctic charr because the effects of duration and magnitude of pressure shocks has not been studied nor has the efficiency of thermal and pressure treatments been compared.

4.2. Growth trials

There was no difference in weight between diploid and triploid juvenile Arctic charr measured over a 597-d period. This is consistent with previous studies (Boulanger, 1991; O'Keefe and Benfey, 1999) in which no growth differential was observed between triploid and diploid female brook trout until the onset of sexual maturation. Our results also corroborate recent studies on other salmonid species in which immature fishes were reared in tanks and in freshwater (Blanc and Vallée, 1999; Sheehan et al., 1999). In Arctic charr, growth performances decrease when dominance hierarchies occur in a tank: growth rates decrease and weight heterogeneity increase (Jobling, 1995). In the present study, diploid and triploid charrs were reared in the same tank. The rapid increase of weight coefficients of variation could indicate that behavioural interactions have reduced fish growth. At the end of the 597-d growth trial, growth rate in diploids was inferior to the expected one in 2-year-old

farmed Arctic charr (Jobling et al., 1993). Further studies will be required to determine whether a dominance of diploids could explain the moderate difference in growth between diploid and triploid Arctic charr we observed. However in brook trout, a species closely related to Arctic charr, O'Keefe and Benfey (1999) did not report any difference of growth between diploids or triploids reared separately or in common.

During the months that preceded the first spawning period, the mean weight of diploid Arctic charr became statistically greater than that of triploids. Thereafter, a sharp decrease occurred in diploid growth rate, and the weight of triploids and diploids became very similar. Increased growth of diploids during the months that preceded spawning could be explained by an effect of gonadal steroids (see Benfey, 1999, for review). Decreased growth of diploids at the onset of reproduction has already been observed by Boulanger (1991) in female brook trout. At the end of the 443-d trial of brood stock growth, triploids did not perform better than diploids in terms of growth and survival, despite a brief advantage for triploids during the onset of the spawning period. Our results support those of Lincoln and Scott (1984) in which no growth differential was observed between diploid and triploid rainbow trout spawners. However, in the same species, Quillet et al. (1988) observed that female triploids performed better than female diploids for both survival and growth. In agreement with Quillet et al. (1988), Sheehan et al. (1999) indicated that all female triploid rainbow trouts grew better than diploid females. However in sub-optimal rearing conditions, all female triploid rainbow trouts exhibited higher mortality and lesser growth than diploids (Ojolick et al., 1995). In triploid Atlantic salmon reared in seawater, growth was better and mortality was higher than in diploids (O'Flynn et al., 1997). During the 443-d growth trial, mortality was important for both diploid and triploid Arctic charr. It cannot be excluded that in more optimal rearing conditions, triploid Arctic charr will perform better than in our experiment.

In triploid salmonids, previous works have not reported ovulated females, but generally a few oocytes have been found in the ovaries of immature fish (Lincoln and Scott, 1984; Benfey and Sutterlin, 1984b). As a result of low endocrine activity in ovary, oocyte development occurs at a very slow rate in triploid females (see Benfey, 1999, for review). Allen and Stanley (1978) had reported the occurrence of spontaneous sterile brook trout associated with several different types of mosaicism 2n/3n, 2n/3n/4n, 2n/3n/4n/5n. Recently, Yamaki et al. (1999) also reported the sterility of a mosaic female Japanese charr. We cannot exclude the occurrence of mosaic individuals in our triploids by disturbance of the divisions of their reproductive cell lines. In other fish families, mature triploid females have already been described. In the cichlid fish, *Oreochromis aureus*, Penman et al. (1987) report that gonadal development is sometimes observed in triploid females. In the cyprinid *Carassius auratus gibelio*, natural triploid populations of gynogenetic females occur in central Europe (see Chourrout, 1982, for review). Despite a triploidy rate of 100 % (measured in samples of 54 fish by an experimental group), we cannot totally exclude the possibility that Arctic charr with developed ovaries identified as triploid were in actual fact diploid females. But the impossibility of obtaining viable embryo using ova of these females and their low fecundity relative to diploid control females indicate that these fish were probably triploid.

In males, our results are consistent with previous reports for experimentally produced triploids in other species. Many authors have reported triploid males to have well-developed testis but most of them did not give spermatozoa. In rainbow trout, a little quantity of watery milt containing motile spermatozoa could be collected from some triploid males but it was impossible to obtain viable embryos using this milt (Lincoln and Scott, 1984). We have not detected an emission of milt in triploid Arctic charr, but we have not practiced microscopic observations to detect the presence of spermatozoa in the urine.

Carcass somatic index (CSI) did not differ between immature triploids, diploid and triploid males of Arctic charr. These results are similar to those for rainbow trout (Chevassus et al., 1983). In both species, diploid mature females have a CSI inferior to that of other fish categories, as reported in rainbow trout (Lincoln and Scott, 1984).

In conclusion, triploid Arctic charr were produced by pressure treatments that did not affect egg viability. Growth and survival did not differ from those of diploids, despite an increase in mortality and a decrease in growth of diploid charrs during the spawning period. The triploids presented similar disruption of gonadal development and functional sterility as described for the other salmonids.

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