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The production of gynogenetic Atlantic salmon, *Salmo salar* L.

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Abstract The production of Atlantic salmon gynogenomes by the combined use of a novel method for sperm irradiation and differently timed high hydrostatic pressure shocks is described. Sperm solutions were exposed to UV irradiation in a temperature-controlled flow-through device. Eggs fertilised with such sperm were exposed to shocks of 9500 psi at 30 min or approximately 7 h after fertilisation in order to produce meiotic and mitotic gynogenomes respectively. Yields of meiotic gynogenomes were generally high (up to 95%); those of mitotic gynogenomes were lower (range 2–20%). Analyses of the offspring by ploidy status and fingerprinting confirmed their gynogenetic origin. Small numbers of mitotic gynogenetic fish were grown on for 2 years in fresh and salt water. S1/S2 ratios were lower in gynogenetic fish and mean age at maturity was greater. Of the presumptive gynogenetic fish subjected to destructive sampling ($n = 87$) all were female.

Key words *Salmo salar* · Gynogenesis
Fingerprinting · Ploidy manipulation

Introduction

Gynogenesis, the induction of maternal-only inheritance, is a potentially rapid method of producing highly inbred animals. F_1 hybrids of known and similar genetic constitution derived from clones would, by virtue of their reduced variability, be more suitable animals in a number of research areas. For instance, they would better enable the differential contributions of genetic and environmental effects in disease control to be analysed. Gynogenomes would also be of immense use in bioassays where their reduced variability could lead to improved test systems and to a reduction in the numbers of animals used.

Gynogenesis trials demand an accurate knowledge both of sperm-irradiation procedures, to fully inactivate the paternal genetic contribution whilst maintaining fertilisation rates, and of ploidy manipulation parameters, to restore diploidy in eggs fertilised with irradiated sperm which would otherwise be haploid. Their success may be examined by a variety of techniques such as differential survival, measurement of ploidy status, and analysis of genetic constitution. Many fish species are particularly suited to gynogenesis since they produce numerous externally fertilised eggs, and in a recent review (Ihssen et al. 1990) the different methodologies used in fish gynogenesis and its potential application in aquaculture and fisheries research were discussed.

Atlantic salmon gynogenomes may be produced by interference with either the second meiotic, or first mitotic, division of fertilised eggs. An ability to suppress the expulsion of the second polar body during the former event by using high hydrostatic pressure shocks had already been achieved in earlier triploid induction trials (Johnstone et al. 1991). In the present paper, the production of Atlantic salmon meiotic and mitotic gynogenomes, using a novel method for the close control of sperm irradiation in combination with differently timed pressure shock treatments, is described.

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Materials and methods

Experimental design

Individual experiments used the eggs of a single female fertilised by the milt of a single male. Eggs were fertilised with either control (non-irradiated) or irradiated milt and divided into groups which were subsequently exposed either to high hydrostatic pressures at the presumed times of the second meiotic and first mitotic divisions or left

untreated. Egg groups fertilised with control sperm were expected to be either diploid, triploid, or tetraploid after, respectively, no exposure, exposure at the meiotic timing, or exposure at the mitotic timing. Similarly, exposed eggs fertilised with successfully irradiated sperm were expected by analogy to be either haploid or to be meiotic or mitotic gynogenomes. In most experimental seasons attempts were made to include all these various treatment groups in order that the subsequent success of the sperm irradiation and hydrostatic treatment protocols could be separately examined.

Sperm irradiation protocols

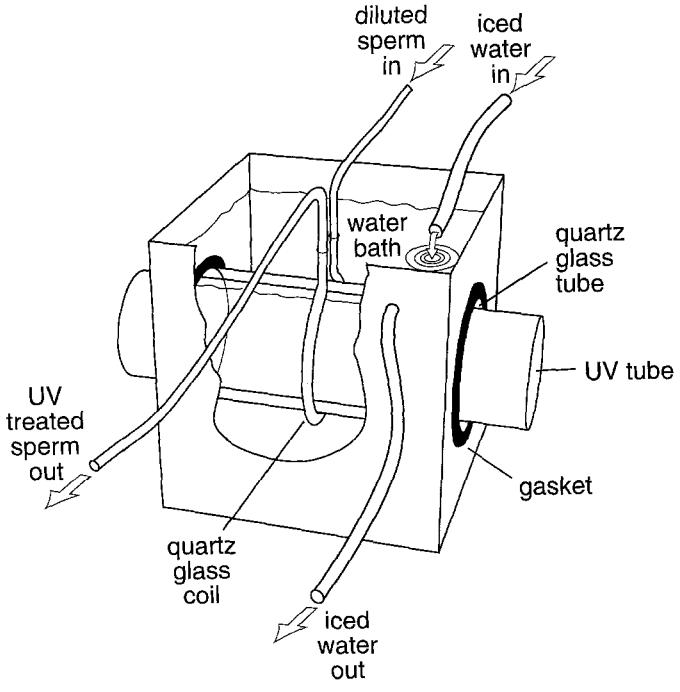
Conventionally in those gynogenesis experiments which use UV irradiation, (e.g., Onozato 1984), sperm is treated by maintaining thin films of diluted, stirred and cooled sperm solutions at some distance (many cms) from germicidal tubes. Treatment times vary between different studies but are commonly of many minutes duration. Initial attempts to treat sperm in this way proved variable in our hands and, because of this, and because of the expectation that the successful fertilisation of large batches of the comparatively large salmon egg would demand large amounts of irradiated sperm, the treatment of sperm in a flow-through device was investigated.

In this study, continually stirred, cooled (on ice) and diluted sperm solutions were pumped at a known rate through a 2-mm internal diameter quartz glass coil positioned at a distance of 1 cm from the external surface of a UV tube emitting at 254 nm (Fig. 1). In order to minimise any heating effects which might have arisen from the close proximity of the sperm to the UV source, the coil was surrounded by a water bath through which an iced water supply was continuously pumped. This enabled the water bath to be maintained below 5 °C at all times. The UV tube was separated from the water in the bath by a quartz glass tube running parallel to its long axis and which was secured to the water bath by two rubber gaskets. All tubing carrying sperm solutions, except that surrounding the UV tube, together with the holding and collecting beakers, was wrapped in aluminium foil to protect the sperm from extraneous exposure and to minimise any potential light-mediated repair mechanisms. In comparison to conventional methods, this approach substitutes long treatments (mins) at low UV intensity for short treatments (s) at high intensity.

In the coil method of sperm irradiation, dose rates can be altered either by adjusting the speed of passage through the coil, or by adjustments to the dilution rate, and hence the opacity, of sperm solutions before treatment at a known and constant pump speed. Preliminary experiments suggested adjustments to the latter to be more important, implying that, at higher concentrations, some individual spermatozoa were being shielded from receiving an adequate dose. This, in turn, suggested that close attention to sperm concentration during irradiation would be necessary to ensure consistency of treatment in different seasons using different males. An attempt was therefore made to maintain constancy of dose rate in different experiments by alterations to spermatocrit (SC), dilutions being made after initial adjustment to a nominal spermatocrit of 30%. It was subsequently established that although, as expected, spermatocrit and sperm density as measured by cytometry were strongly correlated, there was sufficient variability in sperm density between males to lead to differences of sperm concentration during treatment of 50% if spermatocrit alone was used as the measure of sperm concentration. In subsequent seasons, therefore, sperm solutions were exposed at known sperm densities after determination of sperm concentration using haemocytometer slide. During irradiation, sperm were extended in an ice-cold modified Cortland extender (Truscott et al. 1968). At the chosen pump speed (0.75 ml/min), differently diluted sperm solutions were exposed for about 16 s to 10^4 $\mu W/cm^2$.

Following appropriate irradiation treatments (see below), approximately 50% of spermatozoa demonstrated normal activity upon activation with ovarian fluid. In addition, the period of swimming activity was severely reduced. Because of this, and to enable its use in a manner similar to that of normal sperm, irradiated milt solutions were concentrated to approximately half their normal spermatocrit using mild centrifugation (6500 g for 10 s) in the expectation that fertilisation rates would thereby be maximised. Control sperm solutions were similarly handled but were not treated with UV. Eggs were "dry" fertilised after decanting any ovarian fluid. Sperm were activated and fertilisation initiated by the rapid and sequential addition of, firstly, the decanted ovarian fluid and then of 10 °C hatchery water. Time 0 mins was taken as the time of addition of ovarian fluid. Eggs were maintained in a 150-l temperature-controlled water bath (10 ± 0.1°C) at all times prior to the ploidy manipulation treatments.

Fig. 1 Cut away diagram of the coil UV-irradiation device



Ploidy manipulation

The preferred method of interference with the second meiotic division in Atlantic salmon ova, namely, exposure of eggs to high hydrostatic pressure in a specially constructed 2-l vessel, was already known to us as a result of work on triploidy inducing trials [Johnstone et al. (1991), 9 500 psi × 5 min at 30 min after fertilisation]. It was assumed that similar treatments would be effective in suppressing the first mitotic division, that is, in the production of mitotic gynogenomes. Some knowledge was needed, however, of the timings at which it might be appropriate to expose eggs to ploidy manipulation procedures.

The time window for interference with the first mitotic division was initially determined on eggs fertilised with normal sperm, that is by attempted tetraploidy induction, as suggested by Chourrout (1987), and used the anaesthetic chemical nitrous oxide as the ploidy manipulating agent (Johnstone et al. 1989). A period of increased mortality associated with nitrous-oxide treatment was discovered in the interval 7–7.5 h after fertilisation. Although little evidence of tetraploidy was seen by microdensitometric analysis of the survivors in these experiments, eggs fertilised with irradiated sperm were subsequently exposed to hydrostatic-pressure shock treatments at the same timings in order establish their efficacy in producing mitotic gynogenomes.

Egg incubation and ploidy assessment

Following fertilisation, and, where done, any ploidy manipulation procedures, eggs were removed from the laboratory to an ambient-temperature hatchery facility 30 miles west of Aberdeen. Here, they were raised until yolk-sac absorption was nearly complete and they were ready to commence feeding. Dead eggs and alevins were recorded and removed. Survival rates were calculated as the percentage of

animals of normal appearance surviving to this point. Fry were sampled at this time for microdensitometric analysis (Johnstone and Lincoln 1986) by placing in fixative, or for DNA analysis (see below) by freezing on solid CO_2. In some individuals, because the portion of the body posterior to the yold sac was frozen and the anterior portion containing the head and heart was fixed, it was possible to relate ploidy status with genotype. In later trials most putative mitotic gynogenomes were not sampled but were weaned onto diet.

DNA fingerprinting

High-molecular-weight DNA was obtained from the posterior half of the frozen fry after removal of the fins. Fry were homogenised in TEN [100 mM Tris/HCl pH 8.0, 10 mM EDTA, 250 mM NaCl, and 1% (w/v) SDS] buffer and incubated overnight at 50 °C in the presence of proteinase-K (1 mg/ml). DNA was extracted by phenol and chloroform/iso-amyl alcohol, and precipitated with 2-propanol at −20 °C. This precipitate was dissolved in TE (10 mM Tris/HCl pH 7.6, 1 mM EDTA), and again precipitated with sodium acetate and 100% ethanol. The DNA was washed in 70% ethanol, dried and dissolved in TE. DNA samples (20 µg) were digested in a large volume (400 µl) with *Alu*I or *Hae*III (generally 5 units/µg) according to the manufacturer's specifications in the presence of RNAse. DNA was precipitated and dissolved in TE and used directly for electrophoresis. Digested DNA samples (5 µg) were separated on agarose gels and transferred to nylon filters (Hybond-N+, Amersham, UK) using a vacuum-blot system. Radiolabelling of the mini-satellite probe *Per* (Georges et al. 1988) to a high specific activity was performed by multiprime labelling essentially according to Feinberg and Vogelstein (1983). The micro-satellite oligonucleotide probe $(GGAT)_4$ was end-labelled using polynucleotide kinase according to specification. Filters containing *Hae*III-digested DNA were prehybridised in 5 × SSC, 5 mM EDTA, 5 × Denhardt's, 0.1% SDS, 100 µg of denatured *E. coli* DNA, and 50% formamide for 8 h at 42 °C and then hybridised overnight in the presence of a labelled *Per* probe (1×10^6 cpm ml^{-1}). Filters were washed at a high stringency of 0.1 × SSC at 65 °C. Filters to be hybridised with the oligeonucleotide probe $(GGAT)_4$ were prehybridised in 5 × SSC, 5 mM EDTA, 0.1% SDS, 100 µg of *E. coli* DNA at 43°C and then hybridised in the presence of 1×10^6 cpm ml^{-1} of labelled probe. The filters were washed for 30 mins in 4 × SSC at room temperature, followed by 5 mins at 43 °C. Autoradiography was performed at −70 °C using Kodak X-OMAT AR imaging films with intensifying screens. Autoradiographs of fingerprinting profiles were read by a gelscan XL [2.1] (LKB Pharmacia) and converted into multiple alignment plots.

Results

Experiment 1. Effects of dilution rate of sperm solutions during UV irradiation on subsequent gynogenetic yield

Eggs were fertilised with either control or differently diluted sperm solutions exposed to the same UV irradiation treatment (Table 1). Subsequently, the differently fertilised batches were divided and a proportion subjected to hydrostatic pressure shock.

Eggs fertilised with normal sperm (diploid controls) experienced very high survival rates. Similarly fertilised eggs subjected to pressure shock at the meiotic timing also experienced high survival rates, and the triploid rate in a subsample of survivors ($n = 30$) was shown by microdensitometry to be 100%.

Eggs fertilised with sperm diluted 1:10 during UV exposure and not subjected to pressure had a lowered survival rate. Microdensitometric analysis showed all of a sample of normal survivors ($n = 20$) to be diploid. Twenty-two of thirty animals in the comparative pressure shocked group were similarly demonstrated to be triploid, the remainder (26%) being presumptive meiotic gynogenomes.

At the remaining dilutions, none of the unshocked, UV-irradiated sperm-fertilised groups contained survivors of normal appearance at first feeding. The majority of eggs in these groups, although fertile, failed to hatch. In contrast to their comparative unshocked groups, all shocked groups fertilised with milt irradiated at dilutions in excess of 1:20 generated survivors of normal, or near normal, appearance. The yield of these putative meiotic gynogenomes was related to sperm dilution during UV irradiation, the greatest dilution generating yields of 95%, of figure comparable to that seen in the control (triploid yield) trial. All normal survivors analysed in the 1:40 and 1:60 groups ($n = 20$ in both cases) had the same mean erythrocyte density as diploid controls. Four out of fifteen animals in the 1:20 group were adjudged triploid by microdensitometry.

Experiments 2–5. Production of gynogenomes using optimised irradiation treatments

The effect of timing of administration of hydrostatic pressure shock on the survival rate of eggs fertilised with normal and irradiated sperm is summarised in Table 2.

Survival rates in eggs fertilised with normal sperm and not subjected to pressure shock were satisfactory with the exception of experiment 4. On this occasion, for unknown reasons probably connected with fertilisation practice, the control fertilisation rate was very low. Survivors in normally fertilised egg batches treated with pressure at the meiotic timing were 100% triploid indicating the efficacy of the treatment in manipulating ploidy at this timing. Similarly fertilized eggs exposed to pressure around 450–460 min after fertilisation demonstrated poor survival. None of these survivors was confirmed as being solely tetraploid by microdensitometry although some deformed individuals had erythrocytes conforming both to a diploid and a tetraploid DNA content.

With the exception of a single individual in experiment 5, no animals survived to first feeding in the expectedly haploid batches of experiments 2–5; that is, in those eggs fertilised with irradiated sperm and left unshocked. This was despite high rates of survival to eyeing (up to 70%). Those few animals that survived beyond hatch were small eyed and/or poorly pigmented and died in the period between hatch and first feeding.

The groups fertilised with irradiated sperm and shocked at the meiotic timing in these experiments, generated high yields of presumptive meiotic gynogenomes. Normal survivors in these groups were, with two exceptions (exceptions in experiments 3 and 5, total number analysed = 169), demonstrated to be diploid by

Table 1 Experiment 1. The effect of sperm dilution during UV irradiation on % survival to first feeding and ploidy status ( ) in control and pressure-shocked Atlantic salmon

Category	Normal sperm % survival (ploidy)	UV-irradiated sperm (dilution during irradiation)			
		1:10 % survival (ploidy)	1:20 % survival (ploidy)	1:40 % survival (ploidy)	1:60 % survival (ploidy)
Control	99 (all 2n) ($n = 20$)	63.2 (all 2n) ($n = 20$)	0	0	0
Pressure shocked (9.5 K psi × 5′ at 30′ after fertilisation)	95 (all 3n) ($n = 30$)	70.8 (8 2n, 22 3n)	32.3 (15 2n, 4 3n)	67.6 (all 2n) ($n = 20$)	95.2 (all 2n) ($n = 20$)

Table 2 Experiments 2–5. The effects of time of initiation of pressure shock on % survival to first feeding and ploidy status ( ) in Atlantic salmon ova fertilised with normal and irradiated sperm

Experiment no.	Not shocked	Time of initiation of pressure shock (mins after fertilisation)								
		30	400	410	420	430	440	450	460	470
Experiment 2 (wild eggs 9.5 K psi × 5′)										
Normal sperm	[a]	98 (all 3n, $n = 20$)	89	85.5	87.3	58.6	20.7	31.5	4.4 (all 2n, $n = 4$)	–
UV sperm (0.31×10^9/ml; 1:40)	0	86 (all 2n, $n = 20$)	0	0	2	14.6	46.2 (all 2n; $n = 20$)	8.9 (not sampled)	2.6	
Experiment 3 (wild eggs 9.5 K psi × 5′)										
Normal sperm	97.9 (all 2n, $n = 20$)	98.7 (all 3n, $n = 20$)		16.4	85.7	93.5	86.5	31.5	4.4 (all 2n, $n = 4$)	–
UV sperm (0.31×10^9/ml 1:40)	0	98.1 (47 2n, 1 3n)		0	0	0	0	8.9 (not sampled)	2.6	
UV sperm (0.21×10^9/ml, 1:60)		97.1 (all 2n, $n = 48$)		0	0	0	0	7.0 (not sampled)	2.0	–
Experiment 4 (farmed eggs, 9.5 K psi × 5′)										
Normal sperm	2.8 (all 2n, $n = 10$)	0		–	–	–	0	0 (not sampled)	5.6	0
UV sperm (0.31×10^9/ml; 1:40)	0	85.4 (all 2n, $n = 30$)		–	–	–	0	1.4 (not sampled)	2.1	0
Experiment 5 (wild eggs, 8.7 K pi × 10′)										
Normal sperm	99 (all 2n, $n = 20$)	100 (all 3n, $n = 20$)		–	–	–	–	0	–	–
UV sperm (0.21×10^9/ml, 1:60)	1 (one 2n survivor)	64 (22 2n, 1 3n		–	–	–	20.5 (all 2n, $n = 6$)	0.8	0	–

[a] Not performed because of oversight

microdensitometry. Presumptive mitotic gynogenomes were produced when shocks were initiated between 420 and 460 min after fertilisation, the optimal timing apparently varying slightly between the different trials. This coincided with the period of increased sensitivity in normally fertilised eggs to pressure treatment and also to the window of susceptibility as previously indicated by the use of nitrous oxide. All normal mitotic gynogenetic survivors sampled had a diploid erythrocyte DNA complement (Table 2).

Fingerprinting results

Hybridisation patterns obtained with the *Per* probe on DNA of animals fertilised with sperm irradiated at a 1:40 dilution in experiment 1 revealed a number of scorable maternal fragments. Fragments a and b (Fig. 2a) independently segregated in a group of presumptive mitotic gynogenetic individuals. Due to the lack of clearly scorable fragments of paternal origin no such readily justifiable statement could be made concerning the absence of a paternal contribution to the gynogenetic individuals.

Fingerprints depicted as multiple alignment profiles showed that, in the diploid controls, maternal fragments a and b were found in some, but not all, individuals, indicating independent segregation. All the meiotic individuals tested had retained these fragments (Fig. 2b). In contrast, independent inheritance of these same fragments was seen in the mitotic individuals (Fig. 2b).

The degree of relatedness of individuals and the disturbance to expectedly similar patterns in presump-

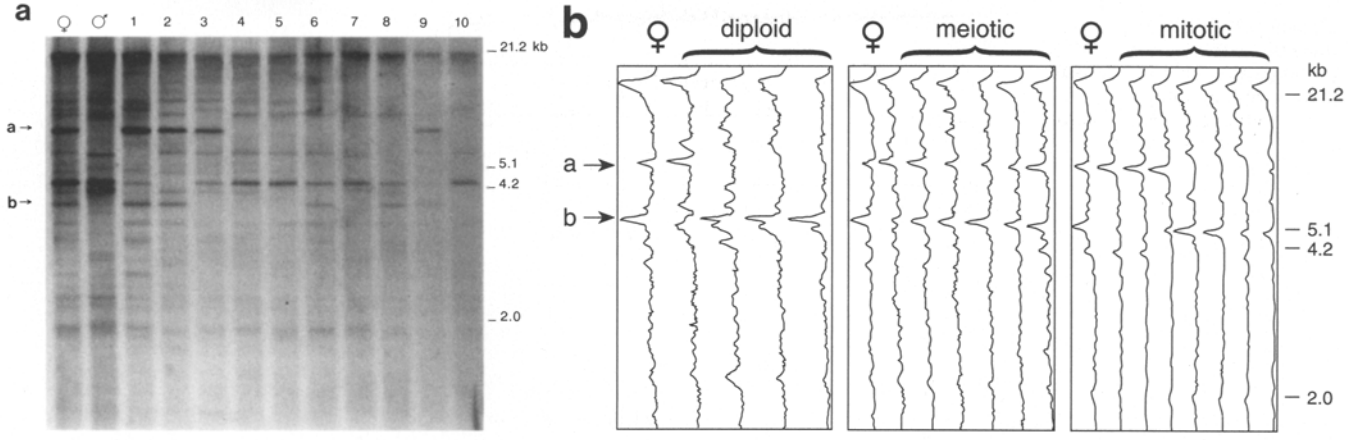


Fig. 2a DNA fingerprints obtained with the minisatellite *Per* probe of the female and male Atlantic salmon parents and ten mitotic gynogenomes produced in experiment 1. **b** Gel-scan alignment profiles of *Per* DNA fingerprints of the female parent and of the diploid, meiotic and mitotic gynogenetic offspring produced in experiment 1

tive gynogenomes that might have resulted from incomplete sperm irradiation was further studied using microsatellite probes. In experiment 3, easily scorable fragments unique to the male parent were rare (Fig. 3a). However, the profiles of the female and of the gynogenomes were closely similar supporting their gynogenetic status (Fig. 3b).

Performance of gynogenomes

The majority of the presumptive mitogenetic fish derived from experiments 3 and 4 were grown on. A failure of the water supply led to the death of all animals in experiment 3. Results with experiment 4 have, to-date, been more successful. After 1 year of fresh-water growth, both control and gynogenetic populations became bimodal in their length frequency distributions, a normal situation for this species. The larger S1 modal groups were transferred to salt water at this time. The growth of gynogenomes throughout the first year was poorer than that of control animals and fewer became S1s as a result (12% vs 61%; Table 3). Similarly, the growth of gynogenomes in the second year was also less good in both the fresh- and salt-water environments (Table 3). The sex ratio in those control animals that have been destructively sampled to-date was essentially equal (8♀:6♂). Of those sampled after 1 year in sea water, all three male animals and one of two females were maturing. In contrast, all the presumptive mitotic gynogenetic fish were female ($n = 7$) and only one was maturing

Fig. 3a DNA fingerprints obtained with the $(GGAT)_4$ microsatellite probe of female and male Atlantic salmon parents, meiotic gynogenetic offspring (*lanes 1–12*) and haploid controls (*13–17*) produced in experiment 3. **b** Gel-scan alignment profiles of $(GGAT)_4$ DNA fingerprints of the female parent and seven meiotic gynogenetic offspring produced in experiment 3

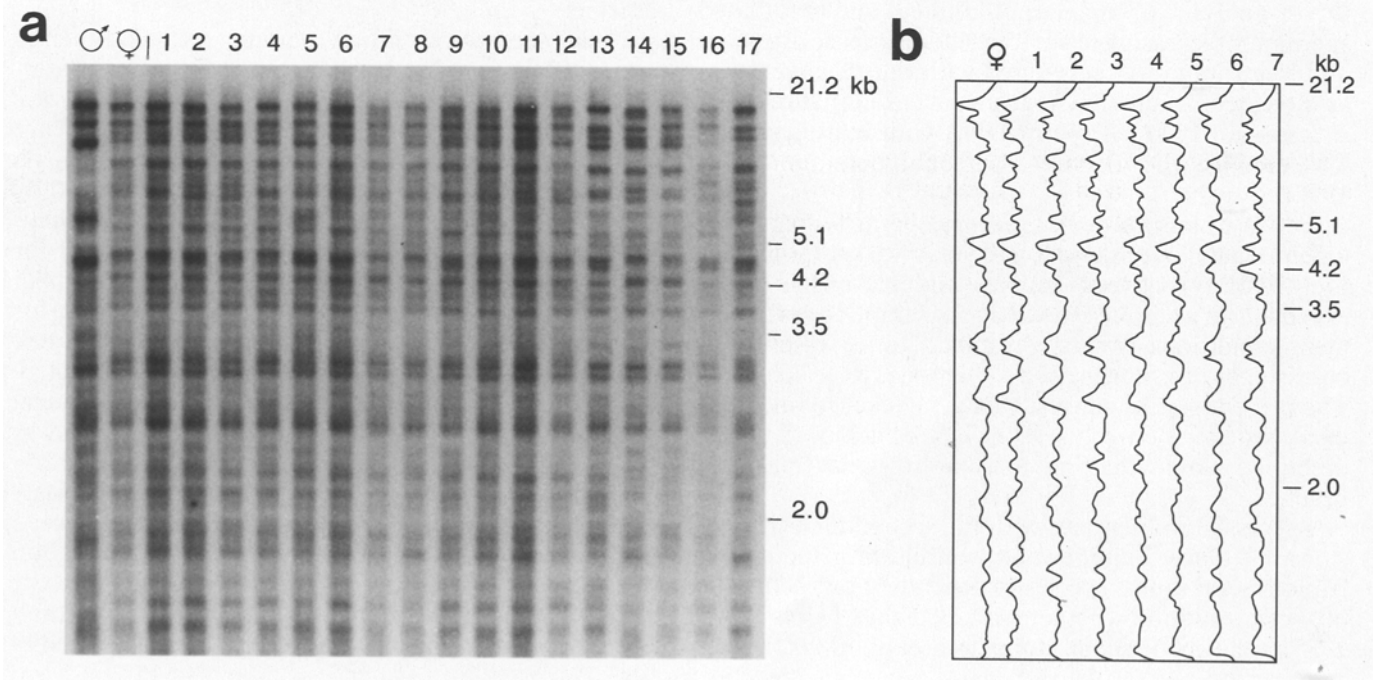


Table 3 Relative growth performance of control and mitotic gynogenetic Atlantic salmon from experiment 3

Age	Parameter	Control		Mitotic gynogenome	
		S1	S2	S1	S2
12 months	Length cm ± SEM (*n*)	10.7 ± 0.1 (19)	7.5 ± 0.2 (12)	10.5 ± 0.3 (8)	6.1 ± 0.2 (60)
24 months	Length cm ± SEM (*n*)	39.1 ± 9.5 (18)	15.6 ± 2.7 (12)	35.4 ± 12.6 (7)	13.9 ± 2.4 (51)
	Weight g ± SEM (*n*)	808.5 ± 50.3 (18)		521.1 ± 50.6 (7)	

at this time. All presumptive meiotic and mitotic gynogenomes that have been destructively sampled from later trials still in progress have also been female ($n = 87$).

Discussion

The results reported here demonstrate that presumptive gynogenetic individuals can be produced in Atlantic salmon and confirm that yield is critically dependent both on the conditions used during sperm irradiation and on the timing of exposure to hydrostatic pressure shock.

Of the two accepted methods of sperm irradiation, gamma ray exposures have been criticised for their capacity to generate fragments of DNA leading to incomplete gynogenetic production (Chourrout 1984). The major drawback to UV irradiation is its poor penetrancy and the results presented here demonstrate that received dose rates, and therefore gynogenetic yield, are markedly affected by sperm concentration during exposure. The performance of the coil device was additionally investigated by analysing the extent to which DNA is degraded by UV treatment. Post-irradiation DNA analysis of differently diluted and irradiated sperm samples, using ultraviolet endonuclease digestion and electrophoresis, correlated with the effective doses as indicated in actual gynogenesis trials in that dilution at less than 1:20 left sperm DNA with relatively little UV damage (D. Penman, personal communication 1992).

Because of its particulate nature, sperm is a relatively difficult medium to sample, and yet, since some measure of variability between animals and seasons is to be expected, an accurate knowledge of sampling and treatment conditions can be presumed to be essential if consistency of gynogenetic production is to be achieved. The present results suggest that a satisfactory measure of consistency between seasons can be achieved by the described close attention to sampling and treatment practice.

Successful interference with the second meiotic division of Atlantic salmon, and optimisation thereby of triploid yield in this species, is dependent on the timing of shock initiation whether with heat shock (Johnstone 1985), anaesthetic shock (Johnstone et al. 1989) or pressure shock (Johnstone et al. 1991 and unpublished results). Essentially for pressure, there is window some 20 min wide (at 10 °C) around 30 min after fertilisation during which the triploid condition can be established. One-hundred-percent triploid rates at high survival rates, that is, high triploid yields, are normal following optimised treatments. Earlier pressure treatments either generate few and/or deformed survivors; later treatments are ineffective. The optimum triploidy generating treatment for pressure shock in this species also generated high yields of meiotic gynogenomes.

Meiotic gynogenetic yield was satisfactorily high (> 64%) when sperm was diluted to 0.31×10^9 ml or greater prior to irradiation in the coil device described. Dilutions less than this either generated survivors in expectedly haploid control groups indicating that some sperm had escaped treatment or caused yields of meiotic gynogenomes to be lowered (experiment 1). Inappropriately irradiated sperm solutions may successfully fertilise eggs but are presumed to give rise to individuals with partially inactivated paternal genomes which abort earlier in the post-fertilisation period, thereby lowering yields. The results of experiment 1 demonstrate the importance of calibration of sperm-irradiation devices in order to determine their optimum working parameters.

These sperm irradiation parameters subsequently proved effective when used in conjunction with later-delivered pressure shocks for the production of mitotic gynogenomes. The optimum timing for interference with first mitosis in Atlantic salmon, namely around $7\frac{1}{2}$ h after fertilisation at 10 °C, is similar to that observed by May et al. (1988) in androgenesis trials with *Salvelinus fontinalis*. This timing is later than that suggested for mitotic gynogenesis by Chourrout (1984) and Quillet et al. (1991) for rainbow trout, or by Onozato (1984) for two Pacific species. With regard to the former, this observation is consistent with the report of Refstie et al. (1977) who showed that, when reared under identical conditions, the two-cell stage of rainbow trout was observable before that of Atlantic salmon.

High yields of meiotic gynogenomes have previously been seen in Atlantic salmon when heat shock was used as the ploidy manipulating agent (Quillet and Gaignon 1990). Yields of mitotic gynogenomes in the present study, though consistently lower and more variable than those of meiotic gynogenomes, were nevertheless some-

times considerable. Lowered yields of mitotic gynogenomes are typical of gynogenesis trials and have been attributed to their expected greater level of inbreeding (Chourrout 1984; Onozato 1984; May et al. 1988; Quillet et al. 1991). Small differences in the timing (10 mins) of pressure shocks generated large differences in mitotic gynogenetic yield in eggs fertilised with irradiated sperm and mitotic gynogenetic yield varied in different seasons. Since there are bound to be inter-individual differences in the potential representation of maternally derived recessives in gynogenetic fish this latter result is perhaps not unexpected. Inter-individual differences in yield have also been seen in previous androgenetic (May et al. 1988) and gynogenetic trials (Quillet et al. 1991).

The small differences in optimal timing may equally have resulted from the difficulty of maintaining a large (150 l) water bath at the required temperature sufficiently accurately as to enable prediction of the best timing of shock initiation with complete confidence. Thus, when temperature was continuously monitored some degree of drift (about 0.1 °C) was observed in most experimental seasons. Small variations in incubation temperature can be expected to have the consequence of shifting the development rate of eggs and, therefore, of the optimum timing for mitotic interference.

We predict that this variability will be most conveniently addressed not by further attention to temperature control but by dividing eggs into equal batches which are shocked sequentially around the expected correct timing. Some, in any given season, will inevitably be shocked at the wrong timing and will generate few gynogenomes. Others will be treated at the right time and will generate sufficient survivors for analysis, although the actual timing may vary in different seasons.

It seems to be the case that the tetraploid condition is more difficult to establish in Atlantic salmon than it is in rainbow trout (Chourrout 1984). This difficulty has an advantage in gynogenesis trails, however, in that it will serve to reduce the potential contamination of gynogenetic groups with tetraploids which would otherwise result if sperm irradiation treatments were suboptimal.

The objectives of fingerprinting in this study were two-fold. Firstly, it was assumed that paternal-specific bands would be detected whose absence in gynogenomes could be demonstrated. Secondly, it was presumed that the pattern of inheritance of female-specific bands would be informative in respect of the differences between meiotic and mitotic gynogenomes and of their differential level of inbreeding. DNA fingerprinting in salmon has proven difficult to interpret due to the high number of bands and the level of background intensity (Taggart and Ferguson 1990; Bentzen et al. 1991). Accordingly, it was decided to use new probes, namely the minisatellite probe *Per* and a number of different microsatellite probes [$(CA_5$, $(TG)_{12}$, $(GACA)_4$ and $(GGAT)_4$].

The *Per* probe was demonstrated to be of use since scorable female-specific bands, seen to segregate in diploid offspring as suggested by Wright (1994), were detected. The limited difference between male and female genotypes demonstrated by this probe is perhaps unusual but may be related to the level of inbreeding of the parents employed. Most commonly, they were drawn from expectedly discrete and small wild spawning populations. The number of scorable fragments was reduced in mitotic gynogenomes indicating their higher level of homozygosity relative to the female parent.

Of the microsatellite probes tested, $(GGAT)_4$ provided the most easily interpretable fingerprint profiles, the others generating unmanageably high numbers of bands. The $(GGAT)_4$ probe would have enabled disturbances in the female genome to be detected if sperm irradiation protocols had been suboptimal. Disturbances of this nature, reflecting incomplete gynogenesis, were observed when UV rays were used to irradiate sperm samples in the attempted production of gynogenetic *Orechromis* spp. (Carter et al. 1991). In the present study, no apparent disturbances to the meiotic gynogenetic genome were observed using the $(GGAT)_4$ probe.

The survival and satisfactory, albeit slow, growth to-date of some of the mitotic gynogenomes produced in experiment 4 offers the promise that certain individuals may be of use in the future generation of cloned lines. Each individual, if gynogenetic, should be female and genetically unique and each, if truly 100% homozygous, has the capability of producing many hundreds, perhaps thousands, of genetically identical eggs. In expectation of their being completely homozygous, the cloning event, via a second generation of gynogenesis, could take place at the meiotic timing. Yields might be expected, therefore, to be improved both because the meiotic event is procedurally easier to suppress and because deleterious genetic material will have been selected out in these individuals. Maintenance of such cloned lines by normal breeding would necessitate the successful sex inversion of some of the stock to the male sex. The potential for this in Atlantic salmon has been previously demonstrated (Johnstone and Youngson 1984) and the combined feasibilities of these approaches in the production of F_1 clones of Atlantic salmon are deserving of further evaluation.

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