ICES Journal of Marine Science, 58: 525–529. 2001
doi:10.1006/jmsc.2000.1019, available online at http://www.idealibrary.com on IDEAL®

Use of sterile triploid Atlantic salmon (*Salmo salar* L.) for aquaculture in New Brunswick, Canada

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Benfey, T. J. 2001. Use of sterile triploid Atlantic salmon (*Salmo salar* L.) for aquaculture in New Brunswick, Canada. – ICES Journal of Marine Science, 58: 525–529.

Induced triploidy is the only effective method currently available for mass production of reproductively sterile salmonids for aquaculture. Repeated studies at the Atlantic Salmon Federation's hatchery (St Andrews, New Brunswick, Canada) have shown only minor differences between triploids and diploids in survival to S1 smolt age (15 months), percentage of the population which became S1 smolts, and mean S1 smolt size. However, a similar study at a commercial hatchery was terminated because of exceptionally high mortality of triploids prior to the start of feeding. Marine growout trials in sea cages showed that triploids grew well in seawater, but had reduced survival rates (leading to a 5–15% reduction in yield at harvest) and high rates of jaw abnormalities. Similar results have been reported elsewhere. Although induced triploidy can be used effectively as a management tool to ensure lack of reproduction, there is at present little support of the aquaculture industry to switch to their large-scale use. In light of fundamental biological differences, it is perhaps naïve to expect triploids to perform as well as diploids using standard culture methods. Triploids should be treated as a new "species" for aquaculture development, beginning with research to determine their optimum rearing requirements.

Key words: aquaculture, sterility, triploidy.

Received 16 October 1999; accepted 15 March 2000.

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Introduction

My aim is to summarize information available on triploid Atlantic salmon (*Salmo salar* L.) as it pertains to their use for aquaculture in New Brunswick, Canada. No original data are presented, but reference is made to published literature containing supporting data. In particular, the reader is referred to the more comprehensive proceedings of two workshops on this topic (Pepper, 1991; Benfey, 1996a).

The interest in triploids arises from the fact that they are sterile. Induced triploidy is by no means the only effective way of sterilizing fish, but it is currently the only method available by which to sterilize large (commercial-scale) numbers of salmon without the use of chemicals that would otherwise affect consumer acceptance of the fish. Some alternative methods for the production of sterile salmon, such as surgical castration, hormonal sterilization, and induced gonadal autoimmunity, have been described elsewhere (Donaldson *et al.*, 1993; Donaldson and Devlin, 1996).

The use of triploids for aquaculture originated with the industry's need to prevent sexual maturation of production fish before they reach market size, because maturing salmon, being chronically stressed, have reduced flesh quality and are more susceptible to disease (Mazeaud *et al.*, 1977). Early maturation of Atlantic salmon is not, however, a concern for New Brunswick's aquaculture industry because precociously mature males are culled prior to seawater transfer of smolts, and because rates of maturation as grilse are exceptionally low. The current interest in triploids is based on the perceived need for "genetic containment" of those fish that inevitably escape from aquaculture facilities, and is therefore driven by forces outside the industry.

Biological effects

As the name implies, triploids have three sets of chromosomes in their somatic cells rather than the normal two sets (diploids). Although there are a few naturally occurring triploid species of fish that exist as all-female

populations with unique reproductive strategies (Purdom, 1984), for most species triploidy is not a natural condition. Tetraploidy has played a role in the evolution of many widespread and economically important groups of fish, including salmonids (Allendorf and Thorgaard, 1984). Triploidy, on the other hand, generally leads to such severe gametogenic impairment that these individuals are sterile (Benfey, 1999). Among the salmonids, triploid males develop much larger gonads than triploid females and often produce functional spermatozoa, but these spermatozoa are aneuploid (Benfey *et al*., 1986) and therefore unable to yield viable offspring upon fertilization of eggs from diploid females (Ueda *et al*., 1987).

Impaired gametogenesis, and its effects on gonadal development, represents one of two fundamental effects of triploidy on basic fish physiology; the second relates to changes in cell size and number. The volume of cell nuclei is increased to accommodate the extra genetic material, and a corresponding increase in cellular volume typically results. However, triploid individuals are no larger than diploids because of a corresponding reduction in cell numbers. An important consequence of increased nuclear and cellular volume is the resulting decrease in the ratio of surface area to volume. This could theoretically affect processes limited by surface area, such as nutrient and metabolite exchange, passive and active ion exchange, and membrane binding of hormones and other messengers. Because of lower cell numbers, the decrease in ratio of surface area to volume applies to entire tissues and organs as well. A second important consequence is that, depending on the shape of the cell and its nucleus, internal transport and diffusion distances may be increased. This could affect processes such as signal transduction from the cell surface to the nucleus, and resultant production and movement of RNA and proteins within and out of the nucleus and cell. Some of these potential disadvantages may be offset by the energetic advantages arising from reduced production and maintenance of cellular membranes and from the smaller relative surface area across which ionic and osmotic gradients must be maintained.

Aside from obvious effects of sterility, a comprehensive review of the literature on the physiology and behaviour of triploids (Benfey, 1999) reveals remarkably little difference from diploids at the whole-animal level. Nonetheless, it may be a mistake to assume that triploids are identical to diploids in their culture requirements, and it has been argued (Benfey, 1996b) that triploids should be treated as ''new species'' for aquaculture development. This is supported by the observation that triploid salmonids perform poorly under conditions of low oxygen availability and/or high oxygen demand by the fish (Quillet and Gagnon, 1990; Johnstone *et al*., 1991; Blanc *et al*., 1992; Simon *et al*., 1993; Ojolick *et al*., 1995; Johnstone, 1996).

Production and identification

Triploids are produced by retention of the second polar body – a package of maternal chromosomes that normally leaves the egg with the completion of meiosis shortly after fertilization. Triploidy is generally induced through either thermal or hydrostatic pressure treatment of eggs within the first hour of fertilization; these treatments disrupt spindle fibres and thereby interfere with the normal movement of chromosomes during meiosis. The ease with which triploid fish can be produced is evidenced by the extensive number of species in which this simple genetic manipulation has been performed successfully (Benfey, 1989). Although thermal treatments are easier to apply to eggs and require less costly equipment, hydrostatic pressure treatments are more easily controlled and therefore preferred. The design of such systems is relatively simple (Benfey *et al*., 1988), and a local company has manufactured several commercial-scale units.

The variables that must be controlled for the successful induction of triploidy are the time after fertilization at which to begin the treatment (which is itself temperature-dependent and therefore measured in °C-min) and the duration and magnitude of the treatment itself. The first successful production of triploid Atlantic salmon was achieved locally (Benfey and Sutterlin, 1984), but the intensity of the treatments described in this early study is now considered to have been excessively high. Good results can be obtained using treatments of 5 min at 9500 psi, beginning 300°C-min after fertilization. This treatment was used successfully in New Brunswick to produce all-triploid populations of five year classes of Atlantic salmon (O'Flynn *et al*., 1997).

With the exception of characteristic jaw abnormalities (described later), triploid salmon are morphologically identical to diploids. Verification is therefore generally done by measuring red blood cell dimensions, either microscopically or with the use of automated particle-sizing instruments (Benfey *et al*., 1984), or by measuring red blood cell DNA content by flow cytometry (Thorgaard *et al*., 1982; Allen, 1983). Flow cytometry is the preferred method because it is rapid and yields unambiguous results, but the equipment is expensive and generally only available through hospital facilities.

All-female populations of triploids are better suited for aquaculture than mixed-sex populations, since females remain sexually immature. Triploid males, although sterile in the sense that they are unable to produce viable offspring, do go through the physiological processes associated with sexual maturation, exhibit normal spawning behaviour, and will mate with diploid females when placed in an appropriate spawning environment (Benfey, 1999). For commercial applications, all-female populations are generally produced

Table 1. Early freshwater survival rate (S in %; fertilization to first-feeding) and S1 smolt rate (sr in %), smolt length (L in cm), weight (W in g), and condition factor (CF; calculated as 100 × weight/length^3) for diploid (2n) and triploid (3n) Atlantic salmon reared at the ASF hatchery in New Brunswick (*p<0.05; ** p<0.01; after O'Flynn *et al.*, 1997).

Year class	S		S1 sr		S1 L		S1 W		S1 CF	
	2n	3n	2n	3n	2n	3n	2n	3n	2n	3n
1990	56.2	42.9	80.8	76.5	18.9	18.8	73.3	72.0	1.06	1.02*
1991	63.4	41.0**	76.2	67.0*	17.0	17.1	52.3	52.9	1.05	1.02**
1992	66.9	67.8	87.6	86.0	18.0	17.9	64.1	62.8	1.08	1.06*
1993	51.7	41.9	94.6	95.5	16.7	17.0	51.5	55.3	1.08	1.02**
1994	24.2	22.0	92.3	87.7	18.8	18.7	70.3	68.6	1.05	1.02**

Table 2. Seawater survival (S in %; smolt transfer to harvest), weight (W in kg), yield (Y in kg per smolt), and incidence of deformities at harvest (I in %) for diploid (2n) and triploid (3n) Atlantic salmon reared at the NBSGA cage site in New Brunswick (**significant at p<0.01; after O'Flynn *et al.*, 1997).

Year class	S		W		Y		I	
	2n	3n	2n	3n	2n	3n	2n	3n
1990	81.5	64.8	2.99	3.20	2.44	2.07	13.6	28.0
1991	65.2	60.4	3.72	3.79	2.43	2.29	1.5	23.2
1992	76.5	66.7	4.13	4.51**	3.16	3.01	4.1	11.8

by first treating a small population of potential broodstock with androgen (e.g. 3 ppm dietary 17α-methyltestosterone for the first 600°C-days after start of feeding), and then crossing the resulting masculinized females (i.e. functional males with the XX-genotype) with normal females. Such a protocol is easily incorporated into triploid production schemes. The only difficulty is to distinguish masculinized diploid females from normal diploid males. This process has been greatly facilitated in some species by the development of Y chromosome-specific genetic markers (Devlin *et al.*, 1991), but such markers have yet to be developed for Atlantic salmon.

Pilot-scale evaluations

As part of a collaborative study between the University of New Brunswick (UNB), the Atlantic Salmon Federation (ASF), and the New Brunswick Salmon Growers' Association (NBSGA), triploid Atlantic salmon were produced every year from 1990 to 1994 at the ASF hatchery in St Andrews, as described above. A variety of domesticated strains was used, with full-sib diploids as controls. Fish were reared to the smolt stage at the hatchery (McGeachy *et al.*, 1994, 1995; O'Flynn *et al.*, 1997), and then transferred to sea cages at the NBSGA demonstration farm in Lime Kiln Bay (McGeachy *et al.*, 1996; O'Flynn *et al.*, 1997). This was followed by the production of triploids in 1994 and 1995 at a commercial hatchery, but these fish were not carried past first-feeding due to unacceptable (and unexplained) high mortality of the triploids (O'Flynn *et al.*, 1997). Survival from fertilization to first-feeding was generally lower for triploids than for diploids at the ASF hatchery, although statistically significant differences were only observed for the 1991 year class (Table 1). There was no statistically significant effect on survival for the remainder of the freshwater production cycle in any year class. There was a general reduction in proportion of the population that became S1 smolts in triploids relative to diploids, but again a statistically significant difference was only observed for the 1991 year class. S1 smolts did not differ in size, but triploids had consistently lower condition factors, giving them more the appearance of wild smolts.

Because of a lack of replicate cages, statistical analysis of survival rates after smolt transfer was not possible. However, survival rates in seawater were consistently lower for triploids than for diploids (Table 2). Surviving triploids were as large as, or larger than, diploids at harvest (Table 2), but considering their higher mortality rates this should not be interpreted as better growth rates. It may be that the smaller triploids were the ones to die prior to harvest, thereby skewing size estimates in favour of larger fish. The actual yields (as kg at harvest per smolt stocked) were 5–15% lower for triploids relative to diploids. Triploids showed a strikingly high

incidence of deformities (Table 2), mainly of the lower jaw. Such lower jaw deformities have been previously described in triploid Atlantic salmon (Sutterlin *et al.*, 1987; Jungalwalla, 1991; Sutterlin and Collier, 1991), but as yet there has been no definitive causal explanation. Fish with lower jaw deformities cannot be sold whole (gutted, head on), but are still suitable for value-added processing (steaks, fillets, etc.).

Research on a comparable scale has been conducted in both Newfoundland (Bay D'Espoir) and British Columbia. In both cases, triploids were on average smaller than diploids at harvest, and showed high rates of jaw deformities (V. A. Pepper and E. M. Donaldson, pers. comm., 1998). Research from Washington State reported similar freshwater growth but poorer growth and survival in seawater for triploid Atlantic salmon compared to diploids; no mention is made of lower jaw deformities (Galbreath *et al.*, 1994; Galbreath and Thorgaard, 1995). More long-term and larger-scale evaluations have been conducted in Scotland (Johnstone *et al.*, 1991; Johnstone, 1996) and Tasmania (Jungalwalla, 1991). Again, the general impression has been one of poorer performance of triploids relative to diploids, especially during seawater growth. Interestingly, a high incidence of lower jaw deformities was also reported in Tasmania (using fish derived from Canadian stocks), but such deformities are rare in Scotland. Scottish triploids, on the other hand, show a higher incidence of cataracts (Wall and Richards, 1992).

Management considerations

Induced triploidy is currently the only practical method by which to ensure sterility of Atlantic salmon produced for aquaculture on a commercial scale. From a strictly management perspective, if sterility needs to be ensured then the present state-of-the-art dictates that triploids should be used. However, until it can be demonstrated that the performance is identical to (or, ideally, better than) that of diploids, the aquaculture industry will likely be opposed to their use. Although a large industry, salmon farming in New Brunswick is currently operating on an exceedingly small (if not negative) profit margin. Imposing the use of triploids at present, based on the data available (5–15% lower yield and high incidence of jaw deformities), could well spell the end of the industry, and any management decisions should therefore not be taken lightly.

Future work

Although pilot-scale aquaculture results with triploids to date have not been good, they certainly have not been disastrous. In fact, if one accepts the suggestion (Benfey, 1996b) to treat triploids as a "new species" for aquaculture development, then the initial results are encouraging. Once optimum rearing conditions are determined, triploid Atlantic salmon may prove to be just as good as, if not better than, diploids as production fish. Specifically, research is needed on determining environmental tolerances and optima (temperature, oxygen, salinity, etc.), nutritional requirements (energy, micronutrients, etc.), disease resistance, and behaviour (aggression, competition with diploids, etc.). The specific problem of lower jaw deformities must be addressed.

Although triploids are sterile, this does not preclude that they may have unexpected ecological effects should they escape or be intentionally released into the environment. Therefore, research is needed on the ecological impacts of triploids in natural environments, specifically, on migratory behaviour (especially in relation to freshwater homing), interaction with conspecific diploids and other species, and life-history characteristics (e.g. lifespan). Although such research could perhaps be addressed through laboratory experiments, controlled releases of all-female triploids would allow a more comprehensive evaluation of ecological impacts. In light of regular escapes of diploid salmon from aquaculture facilities, such an experiment should not have any added risk.

Techniques other than induced triploidy can theoretically be used to sterilize fish, and perhaps better methods can be developed to do this on a commercial scale with salmon.

Acknowledgements

This review was first presented at the Fisheries and Oceans Canada Workshop on Interaction between Wild and Farmed Atlantic Salmon in the Maritime Provinces (Moncton, Canada, 30 November to 4 December 1998), and published as Department of Fisheries and Oceans Research Document 98/166. Most of the New Brunswick data were collected by Anne McGeachy while an MSc student in Biology at University of New Brunswick. I am grateful to her, as well as to Gerry Friars and Fiona O'Flynn (both formerly with the Atlantic Salmon Federation (ASF)), for their critical role in this collaborative study. The contributions of ASF and the New Brunswick Salmon Growers' Association (NBSGA) fish culture staff are also acknowledged. Funding came from the National Research Council (Industrial Research Assistance Program), Department of Fisheries and Oceans (Atlantic Fisheries Adjustment Program), Atlantic Canada Opportunities Agency, and Natural Sciences and Engineering Research Council. Additional unpublished information was supplied at short notice by Ed Donaldson and Bob Devlin (British Columbia), Vern Pepper (Newfoundland), and Ray Johnstone (Scotland). As always, I am grateful for their insight and open sharing of information. Jeff Hutchings (Dalhousie University) and Vern Pepper provided useful comments on an earlier version of the manuscript.

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