ICES CM 2009/Q:11

Triploid Atlantic salmon: current status and future prospects

Tillmann J. Benfey

Department of Biology, University of New Brunswick
Fredericton, New Brunswick, Canada E3B 5A3
(benfey@unb.ca)

Abstract

The concept of using induced triploidy as a means of providing sterile fish for aquaculture and fisheries management is not new, having been first suggested over 30 years ago. Triploid Atlantic salmon were first produced almost 25 years ago, and they have been evaluated in the European, North American and Australian aquaculture industries. Through this work it has been demonstrated that it is easy to mass produce triploid salmon and that, when combined with simple and proven methods for producing all-female populations, triploidy is highly effective at suppressing gonadal development in Atlantic salmon. However, aside from Tasmania, there is currently no use of triploid Atlantic salmon in commercial aquaculture. This paper will review the development of triploidy as a management tool and outline some of the limitations of triploid performance that have influenced the decisions of industry not to adopt this technology. Finally, suggestions will be made for how to approach genetic and husbandry improvements to enhance the potential of triploid Atlantic salmon in commercial culture.

Triploidy has been induced in many species of fish, generally with the goal of providing reproductively sterile populations for aquaculture. The production, identification and characteristics of triploid fish have been extensively reviewed, as has the rationale for their use as a management tool in aquaculture (Pandian and Koteeswaran, 1998; Benfey, 1999; Felip et al., 2001; Hulata, 2001; Maxime, 2008; Benfey, 2009; Piferrer et al., 2009). Triploids are sterile because of the inability of three sets of homologous chromosomes to progress through recombination during the first meiotic division. Most triploid cells fail to complete meiosis, and those that do are typically aneuploid. Triploid males often still undergo substantial gonadal development, with normal endocrine profiles and reproductive behaviours, whereas triploid females retain the characteristics of immature fish throughout their lives. The most effective use of triploids in aquaculture is therefore in combination with simple endocrine manipulations leading to the production of all-female populations.

The first reported production of triploid Atlantic salmon (*Salmo salar*) was published over 60 years ago, albeit in very low numbers and as a hybrid with brown trout (*S. trutta*) (Svärdson, 1945). It was another 30 years before further attempts were made to produce triploid Atlantic salmon, using methods that ultimately proved unsuccessful (Lincoln et al., 1974; Allen and Stanley 1979). Success followed 10 years later (Benfey and Sutterlin, 1984a; Bolla and Refstie, 1985; Johnstone, 1985; Johnstone et al., 1989; Quillet and Gaignon, 1990), leading to the pilot-scale evaluation of triploid Atlantic salmon for aquaculture in Scotland (Johnstone et al., 1991; Johnstone, 1993, 1996; McCarthy et al., 1996), Atlantic Canada (Friars and Benfey, 1991; Sutterlin and Collier, 1991; McGeachy et al., 1995, 1996; Pepper et al., 1996; O'Flynn et al., 1997; Benfey, 2001; Friars et al., 2001), Tasmania (Jungalwalla, 1991), the USA (Galbreath et al., 1994; Galbreath and Thorgaard, 1995), Ireland (Cotter et al., 2000; Wilkins et al., 2001; Cotter et al., 2002) and Norway (Oppedal et al., 2003). Results from these trials were mixed. Triploids tended to grow well, even occasionally outperforming diploids when the latter started to mature, but they often had reduced survival compared to diploids. Mortalities were highest during egg incubation and first feeding, when they have minimal economic impact, and during the marine phase when economic impact is greatest. As a result, salmon farmers in the UK, Norway and Canada showed no interest in adopting triploidy as a management tool, and the use of triploidy in commercial farming of Atlantic salmon is currently limited to Tasmania where there is a high incidence of early sexual maturation of farmed fish as grilse.

A great deal of research has been conducted on triploid Atlantic salmon to determine how they differ from diploids, largely focused on identifying any unique culture requirements. Where comparable data exist, triploid Atlantic salmon show the same general characteristics as do triploids of other species (Benfey, 1999; Maxime, 2008; Piferrer et al., 2009). They have larger red blood cells than diploids, but their numbers are reduced to maintain a similar haematocrit and blood haemoglobin content (Benfey and Sutterlin, 1984b; Sadler et al., 2000a; Cogswell et al., 2002). They do not differ from diploids in their oxygen consumption rates (Benfey and Sutterlin, 1984c), haemoglobin-oxygen binding affinity (Sadler et al., 2000a), aerobic swimming ability (Cotterell and Wardle, 2004; Lijalad and Powell, 2009) or stress response (Sadler et al., 2000a, 2000b). They occasionally have lower jaw and gill deformities (Sutterlin et al., 1987; Sadler et al., 2001) which can affect their ability to recover from exhaustive exercise (Lijalad and Powell, 2009). Triploid Atlantic salmon have less endurance in prolonged swimming tests (Cotterell and Wardle, 2004), suggesting a reduced anaerobic capacity. However, in the absence of jaw deformities, they appear to recover more quickly from exhaustive exercise than diploids (Lijalad and Powell, 2009), a characteristic shared with brook charr (*Salvelinus fontinalis*) (Hyndman et al., 2003a).

A reduced ability to sustain anaerobic metabolism is not likely to be of relevance under typical aquaculture conditions, and nothing else about the physiology of triploid Atlantic salmon as summarized above points to any deficiencies in aquaculture potential. One of the more commonly identified limitations of triploid salmonids is a reduced ability to survive chronic exposure to what would be considered sub-lethally high (for diploids) temperature (Benfey, 1999; Hyndman et al., 2003b; Maxime, 2008). This may reflect lower blood oxygen carrying capacity (Graham et al., 1985), as is also apparent for chinook salmon

(*Oncorhynchus tshawytscha*) (Bernier et al., 2004), which would become problematic at higher temperatures due to increased metabolic oxygen demand by the fish as oxygen solubility decreases with rising temperature. Arguably the most significant recent finding with respect to triploid limitations in aquaculture in this regard is that they have lower thermal optima than diploids, which could explain why they tend not to perform as well as diploids when reared using thermal regimes optimized for the latter (Atkins and Benfey, 2008).

Many fish farmers feel that triploid Atlantic salmon are more susceptible to disease than diploids, but there is no published scientific evidence to support this. Research on complement activity in triploid Atlantic salmon suggests that they may be slightly disadvantaged compared to diploids in their ability to withstand exposure to bacterial pathogens (Langston et al., 2001). The only published study of disease resistance in triploid Atlantic salmon found them to be no different from diploids in their susceptibility to bacterial kidney disease, but this study used triploids and diploids from different families (Bruno and Johnstone, 1990). Studies with other salmonids have generally found no effect of triploidy on complement and phagocyte activity, vaccine efficacy or disease resistance (Benfey, 1999; Maxime, 2008), although Jhingan et al. (2003) did find triploid coho salmon (*Oncorhynchus kisutch*) to be less resistant than diploids to vibriosis from *Vibrio anguillarum*.

In terms of their behaviour, triploid Atlantic salmon were found to be less aggressive than diploids in one study (Carter et al., 1994) but not in another (O’Keefe and Benfey, 1997), likely due to differences in fish size and experimental design. Studies with other salmonids have failed to find a consistent effect of triploidy on aggressiveness or the establishment of feeding hierarchies (O’Keefe and Benfey, 1999; Garner et al., 2008). But in any case, for the sake of inventory management, triploids are not likely to be reared together with diploids in commercial culture.

It is unclear how a second maternal set of chromosomes affects the ability to select for improved production traits in triploids. Given that they are sterile, triploids themselves obviously cannot form the basis of any selection program. Ideally one would select based on diploid performance, making triploids from the same parents that give the best performing diploids, but a study with triploid Atlantic salmon concluded that selection programs based on diploid performance may not yield the best performing triploids (Friars et al., 2001). This conclusion is not supported by research with other salmonids, which have generally found little evidence of family X ploidy interactions (Blanc and Vallée, 1999; Johnson et al., 2004; Shrimpton et al., 2007; Chiasson et al., 2009). However, the doubled maternal genome does apparently affect phenotypic expression of important production traits in unpredictable ways that may still affect selection programs (Johnson et al., 2007).

In conclusion, a review of the literature indicates that there are no dramatic effects of triploidy on Atlantic salmon production traits in aquaculture, aside from their sterility. Their oft reported reduced performance may reflect sub-optimal rearing conditions or the need to use different strains of fish. For all the research done evaluating triploid Atlantic salmon in Atlantic Canada, there is no information on how triploids of the best performing diploid strains compare to their diploid siblings. Future research should evaluate the performance of triploids derived from the best performing production strains, and should focus on defining the optimum rearing environment for triploids.

References

Allen S.K., Jr., Stanley J.G. 1979. Polyploid mosaics induced by cytochalasin B in landlocked Atlantic salmon *Salmo salar*. Trans. Amer. Fish. Soc. 108: 462-466.

Atkins M.E., Benfey T.J. 2008. Effect of acclimation temperature on routine metabolic rate in triploid salmonids. Comp. Biochem. Physiol. 149A: 157-161.

Benfey T.J. 1999. The physiology and behaviour of triploid fishes. Rev. Fish. Sci. 7: 39-67.

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

Benfey T.J. 2009. Producing sterile and single-sex populations of fish for aquaculture. In: New Technologies in Aquaculture: Improving Production Efficiency, Quality and Environmental Management (G. Burnell and G Allan, eds.) Woodhead Publishing Ltd., Cambridge, UK.

Benfey T.J., Sutterlin A.M. 1984a. Triploidy induced by heat shock and hydrostatic pressure in landlocked Atlantic salmon (*Salmo salar* L.). Aquaculture 36: 359-367.

Benfey T.J., Sutterlin A.M. 1984b. The haematology of triploid landlocked Atlantic salmon, *Salmo salar* L. J. Fish Biol. 24: 333-338.

Benfey T.J., Sutterlin A.M. 1984c. Oxygen utilization by triploid landlocked Atlantic salmon (*Salmo salar* L.). Aquaculture 42: 69-73.

Bernier N.J., Brauner C.J., Heath J.W., Randall D.J. 2004. Oxygen and carbon dioxide transport during sustained exercise in diploid and triploid chinook salmon (*Oncorhynchus tshawytscha*). Can. J. Fish. Aquat. Sci. 61: 1797-1805.

Blanc J.M., Vallée F. 1999. Genetic variability of farming performances in some salmonid species and hybrids modified by triploidization. Cybium 23 (Suppl. 1): 77-88.

Bolla S., Refstie T. 1985. Effect of cytochalasin B on eggs of Atlantic salmon and rainbow trout. Acta Zool. (Stockholm) 66: 181-188.

Bruno D.W., Johnstone R. 1990. Susceptibility of diploid and triploid Atlantic salmon *Salmo salar* L., to challenge by *Renibacterium salmoninarum*. Bull. Eur. Assoc. Fish Pathol. 10: 45-47.

Carter C.G., McCarthy I.D., Houlihan D.F. 1994. Food consumption, feeding behaviour, and growth of triploid and diploid Atlantic salmon, *Salmo salar* L., parr. Can. J. Zool. 72: 609-617.

Chiasson M., Pelletier C.S., Benfey T.J. 2009. Triploidy and full-sib family effects on survival and growth in juvenile Arctic charr (*Salvelinus alpinus*). Aquaculture 289: 244–252.

Cogswell A.T., Benfey T.J., Sutterlin A.M. 2002. The hematology of diploid and triploid transgenic Atlantic salmon (*Salmo salar*). Fish Physiol. Biochem. 24: 271-277.

Cotter D., O'Donovan V., O'Maoileidigh N., Rogan G., Roche N., Wilkins N.P. 2000. An evaluation of the use of triploid Atlantic salmon (*Salmo salar* L.) in minimising the impact of escaped farmed salmon on wild populations. Aquaculture 186: 61-75.

Cotter D., O'Donovan V., Drumm A., Roche N., Ling E.N., Wilkins N.P. 2002. Comparison of freshwater and marine performances of all-female diploid and triploid Atlantic salmon (*Salmo salar* L.). Aquacult. Res. 33: 43-53.

Cotterell S.P., Wardle C.S. 2004. Endurance swimming of diploid and triploid Atlantic salmon. J. Fish Biol. 65 (Suppl. A): 55-68.

Felip A., Zanuy S., Carillo M., Piferrer F. 2001. Induction of triploidy and gynogenesis in teleost fish with emphasis on marine species. Genetica 111: 175-195.

Friars G.W., Benfey T.J. 1991. Triploidy and sex-reversal in relation to selection in the Salmon Genetics Research Program. Can. Tech. Rep. Fish. Aquat. Sci. 1789: 81-87.

Friars G.W., McMillan I., Quinton V.M., O'Flynn F.M., McGeachy S.A., Benfey T.J. 2001. Family differences in relative growth of diploid and triploid Atlantic salmon (*Salmo salar* L.). Aquaculture 192: 23-29.

Galbreath P.F., St. Jean W., Anderson V., Thorgaard G.H. 1994. Freshwater performance of all-female diploid and triploid Atlantic salmon. Aquaculture 128: 41-49.

Galbreath P.F., Thorgaard G.H. 1995. Saltwater performance of all-female triploid Atlantic salmon. Aquaculture 138: 77-85.

Garner S.R., Madison B.N., Bernier N.J., Neff B.D. 2008. Juvenile growth and aggression in diploid and triploid Chinook salmon *Oncorhynchus tshawytscha* (Walbaum). J. Fish Biol. 73: 169-185.

Graham M.S., Fletcher G.L., Benfey T.J. 1985. Effect of triploidy on blood oxygen content of Atlantic salmon. Aquaculture 50: 133-139.

Hulata G. 2001. Genetic manipulations in aquaculture: a review of stock improvement by classical and modern technologies. Genetica 111: 155-173.

Hyndman C.A., Kieffer J.D., Benfey T.J. 2003a. The physiological response of diploid and triploid brook trout to exhaustive exercise. Comp. Biochem. Physiol. 134A: 167-179.

Hyndman C.A., Kieffer J.D., Benfey T.J. 2003b. Physiology and survival of triploid brook trout following exhaustive exercise in warm water. Aquaculture 221: 629-643.

Jhingan E., Devlin R.H., Iwama G.K. 2003. Disease resistance, stress response and effects of triploidy in growth hormone transgenic coho salmon. J. Fish Biol. 63: 806-823.

Johnson R.M., Shrimpton J.M., Heath J.W., Heath D.D. 2004. Family, induction methodology and interaction effects on the performance of diploid and triploid chinook salmon (*Oncorhynchus tshawytscha*). Aquaculture 234: 123-142.

Johnson R.M., Shrimpton J.M., Cho G.K., Heath D.D. 2007. Dosage effects on heritability and maternal effects in diploid and triploid Chinook salmon (*Oncorhynchus tshawytscha*). Heredity 98: 303-310.

Johnstone R. 1985. Induction of triploidy in Atlantic salmon by heat shock. Aquaculture 49: 133-139.

Johnstone R. 1993. Maturity control in Atlantic salmon. In: Recent Advances in Aquaculture IV (J.F. Muir and R.J. Roberts, eds.), p. 99-105. Oxford: Blackwell Scientific Publications.

Johnstone R. 1996. Experience with salmonid sex reversal and triploidization technologies in the United Kingdom. Bull. Aquacult. Assoc. Can. 96–2: 9-13.

Johnstone R., Knott R.M., MacDonald A.G., Walsingham M.V. 1989. Triploidy induction in recently fertilized Atlantic salmon ova using anaesthetics. Aquaculture 78: 229-236.

Johnstone R., McLay H.A., Walsingham M.V. 1991. Production and performance of triploid Atlantic salmon in Scotland. Can. Tech. Rep. Fish. Aquat. Sci. 1789: 15–36.

Jungalwalla P.J. 1991. Production of non-maturing Atlantic salmon in Tasmania. Can. Tech. Rep. Fish. Aquat. Sci. 1789: 47–71.

Langston A.L., Johnstone R., Ellis A.E. 2001. The kinetics of the hypoferraemic response and changes in levels of alternative complement activity in diploid and triploid Atlantic salmon, following injection of lipopolysaccharide. Fish Shellfish Immunol. 11: 333-345.

Lijalad M., Powell M.D. 2009. Effects of lower jaw deformity on swimming performance and recovery from exhaustive exercise in triploid and diploid Atlantic salmon *Salmo salar* L. Aquaculture 290: 145-154.

Lincoln R.F., Aulstad D. Grammeltvedt A. 1974. Attempted triploid induction in Atlantic salmon (*Salmo salar*) using cold shocks. Aquaculture 4: 287-297.

Maxime V. 2008. The physiology of triploid fish: current knowledge and comparisons with diploid fish. Fish Fisheries 9: 67-78.

McCarthy I.D., Carter C.G., Houlihan D.F., Johnstone R., Mitchell A.I. 1996. The performance of all-female diploid and triploid Atlantic salmon smolts on transfer together to sea water. J. Fish Biol. 48: 545-548.

McGeachy S.A., Benfey T.J., Friars G.W. 1995. Freshwater performance of triploid Atlantic salmon (*Salmo salar*) in New Brunswick aquaculture. Aquaculture 137: 333-341.

McGeachy S.A., O'Flynn F.M., Benfey T.J., Friars G.W. 1996. Seawater performance of triploid Atlantic salmon in New Brunswick aquaculture. Bull. Aquacult. Assoc. Can. 96–2: 24-28.

O'Flynn F., McGeachy S.A., Friars G.W., Benfey T.J., Bailey J.K. 1997. Comparisons of cultured triploid and diploid Atlantic salmon (*Salmo salar* L.). ICES J. Mar. Sci. 54: 1160-1165.

O'Keefe R.A., Benfey T.J. 1997. The feeding response of diploid and triploid Atlantic salmon and brook trout. J. Fish Biol. 51: 989-997.

O'Keefe R.A., Benfey T.J. 1999. Comparative growth and food consumption of diploid and triploid brook trout (*Salvelinus fontinalis*) monitored by radiography. Aquaculture 175: 111-120.

Oppedal F., Taranger G.L., Hansen T. 2003. Growth performance and sexual maturation in diploid and triploid Atlantic salmon (*Salmo salar* L.) in seawater tanks exposed to continuous light or simulated natural photoperiod. Aquaculture 215: 145-162.

Pandian T.J., Koteeswaran R. 1998. Ploidy induction and sex control in fish. Hydrobiologia 384: 167-243.

Pepper V.A., Sutterlin A.M., Nicholls T., Collier C. 1996. Newfoundland experience with development of all-female and non-reproductive Atlantic salmon for marine aquaculture. Bull. Aquacult. Assoc. Can. 96–2: 14-23.

Piferrer F., Beaumont A., Falguière J.-C., Flajšhans M., Haffray P., Colombo L. 2009. Polyploid fish and shellfish: Production, biology and applications to aquaculture for performance improvement and genetic containment. Aquaculture 293: 125-156.

Quillet E., Gaignon J.L. 1990. Thermal induction of gynogenesis and triploidy in Atlantic salmon (*Salmo salar*) and their potential interest for aquaculture. Aquaculture 89: 351-364.

Sadler J., Wells R.M.G., Pankhurst P.M., Pankhurst N.W. 2000a. Blood oxygen transport, rheology and haematological responses to confinement stress in diploid and triploid Atlantic salmon, *Salmo salar*. Aquaculture 184: 349-361.

Sadler J., Pankhurst N.W., Pankhurst P.M., King H. 2000b. Physiological stress responses to confinement in diploid and triploid Atlantic salmon. J. Fish Biol. 56: 506-518.

Sadler J., Pankhurst P.M., King H.R. 2001. High prevalence of skeletal deformity and reduced gill surface area in triploid Atlantic salmon (*Salmo salar* L.). Aquaculture 198: 369-386.

Shrimpton J.M., Sentlinger A.M.C., Heath J.W., Devlin R.H., Heath D.D. 2007. Biochemical and molecular differences in diploid and triploid ocean-type chinook salmon (*Oncorhynchus tshawytscha*) smolts. Fish Physiol. Biochem. 33: 259-268.

Sutterlin A.M., Collier C. 1991. Some observations on the commercial use of triploid rainbow trout and Atlantic salmon in Newfoundland, Canada. Can. Tech. Rep. Fish. Aquat. Sci. 1789: 89-96

Sutterlin A.M., Holder J., Benfey T.J. 1987. Early survival rates and subsequent morphological abnormalities in landlocked, anadromous and hybrid (landlocked x anadromous) diploid and triploid Atlantic salmon. Aquaculture 64: 157-164.

Svärdson G. 1945. Chromosome studies on Salmonidae. Rep. Swed. State Inst. Fresh-water Fish. Res. Inst., Drottningholm 23: 151p.

Wilkins N.P., Cotter D, O'Maoileidigh N. 2001. Ocean migration and recaptures of tagged, triploid, mixed-sex and all-female Atlantic salmon (*Salmo salar* L.) released from rivers in Ireland. Genetica 111: 197-212.