

The potential for restocking using all-female triploid brown trout to avoid genetic impact upon native stocks

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Background

The aim of this note is to draw together information on the potential for the use of all-female triploid brown trout for stocking in situations where there is concern about the possible genetic effects of introducing fertile fish.

Resistance to the use of triploids in the past has been based on three issues:-

- i. Arguments regarding the need and whether there are in fact any real genetic "risks" associated with introductions of fertile fish.
- ii. Concern that the triploid fish might have an adverse effect on native stocks through behavioural interactions, predation on eggs etc; and
- iii. Concern about whether triploids are "genetically modified organisms".

This note concentrates on the last two of these areas of potential concern, other potential benefits and drawbacks, and on the logistics of stocking using all-female triploids.

Why all female triploids?

Triploids are infertile and thus remove the risk of genetic interaction with wild stocks in waters where they are introduced.

Male triploids may still develop functional gonad tissue and may participate in spawning behaviour, which could interfere with reproduction of wild stocks.

Female triploids do not develop mature gonads and do not exhibit spawning behaviour. This reduces the potential for harmful interactions with spawning wild fish, and increases somatic growth and over-winter survival.

For these reasons stocking with triploids should be with females only.

Production of triploids.

Developing eggs are rendered triploid by applying a pressure, heat or chemical shock at the time of the second meiotic division (in the first hour after fertilization), causing retention of the polar body in the egg (Crozier and Moffett 1989; Purdom 1983). Chemical and heat shock treatments have given mixed results with a variable proportion of diploids remaining among those successfully transformed to triploids, and pressure shock treatment is generally the method of choice nowadays. Lincoln (1996) reported on tests at Allenbrook Trout Farm which showed a 100% induction of triploids and a very high survival throughout the incubation and first feeding stages. Survival to four weeks after first feeding was 71.1%

compared to 69.8% for untreated controls. Subsequent experience has shown that triploids generally have a slightly lower early survival, as alevins, than diploids.

Triploids can also be produced by crossing tetraploids with diploids. Sheehan et al (1999) suggested that, as this method can produce guaranteed 100% triploids, it could be useful for situations where introduction of any fertile diploids is totally unacceptable.

How do triploids differ from diploid trout?

Apart from having three sets of chromosomes, as opposed to the usual two in trout, there are a number of other relevant differences.

Many organs and tissues have larger but fewer cells in triploids, including the brain, muscle, retina, liver and kidney (Benfey, 1999). This appears to arise because the extra set of chromosomes dictates an increase in cell nucleus dimensions which in turn affects overall cell size. However, this rather fundamental difference appears to have remarkably little knock-on effect upon physiology, behaviour and general performance. Development rates appear very similar, until the onset of sexual maturity in diploids. Diet utilisation and energetics appear unaffected. Triploids are generally less aggressive than diploids, which leads to poorer performance when the two are reared together in intensive culture - but these differences disappear when the two are reared separately.

Stillwell and Benfey (1997) found no difference in the swimming ability of diploid and triploid brook trout (*Salvelinus fontinalis*).

Reduced gonadal growth in triploids may allow increased energy allocation to somatic growth, but this may be largely offset by diminished levels of steroids which have an anabolic effect. Over-winter survival of triploids appears higher than that of equivalent mature diploids, and their appearance is different reflecting this non-mature status. The muscle pigmentation in triploids remains strong compared to maturing diploid females, which shift muscle canthaxanthin to the developing eggs.

It is difficult to differentiate visually between triploids and diploids, particularly when both are immature. Diploids will develop spawning coloration, and the males develop kypes, when they mature whereas triploids will maintain their non-mature appearance. There are a number of ways they can be differentiated in the laboratory, the usual one being by examination of the nuclei of red blood cells taken from fry (Lincoln 1996).

Some field experiences with triploids.

Dillon *et al* (2000) stocked equal numbers of tagged diploid and (mixed sex) triploid rainbow trout into 18 streams in Idaho. A total of 5400 of each were released, and total returns to anglers catches were 931 triploids and 918 diploid. Most were reported in the year of release; only 29 diploids and 23 triploids were reported in the following year. Thus in this case the performance of the two types was similar, with apparently low over-winter survival of both. The authors suggested that they had used a highly-domesticated strain of fish and that low over-winter survival was to be expected.

The growth of mixed-sex diploid (MSD), all female diploid (AFD) and all female triploid (AFT) rainbow trout in raceways was studied by Sheehan *et al* (1999). The three groups were reared separately, and the feeding regime was adjusted each month according to the weight of fish in each raceway. After 265 days the daily weight gain averaged 1.6 g for MSD, 1.8 g for AFD, and 2.4 g for AFT. The authors suggested that the weight gain of the AFT group would have been higher except that it included about 13% diploid females due to partial failure of the triploid treatment (heat shock). An important point here is that the groups of fish were reared separately. Galbreath *et al* (1994), working with Atlantic salmon, noted superior growth in triploids reared in the absence of diploids which disappeared if triploids and diploids were reared together.

An EU AIR project was conducted to examine the scope for use of triploid Atlantic salmon in farms to remove the risk of genetic interactions between escapees and wild stocks (Anon 1999). Survival to the smolt stage was similar for diploids and triploids, as were somatic growth, swimming performance of product quality. However, overall yields were 10-15% lower for triploids due to reduced seawater survival.

Ray Hill, fisheries manager at the Houghton Club in Stockbridge, produces and supplies triploid trout to several other fisheries as well as using them on his own. He started using them to improve over-winter survival of large stock fish (to be released at 2 lb+) which otherwise (since the banning of the use of malachite) exhibited a high level of infection with *Saprolegnia* associated with sexual maturation; on occasions he would lose in excess of half the stock. With triploids, losses are typically less than 1%. The triploids feed more reliably over winter, and so grow more evenly. An experiment on the Itchen involved stocking in the autumn with triploid fish of 300g as an alternative to larger fish the following spring. Survival and growth over winter were good and the fish were free-rising early in the season, and were considered a more reliable contribution to early-season catches than fish just stocked. Ray reports that anglers consider the triploids as a stronger fighter when hooked, but this may be due at least partly to better condition rather than being triploid *per se*. Observations on his own water suggest that triploids do not accompany spawning fish onto the shallow redd areas but remain in their normal feeding areas throughout the autumn and winter.

Reliability of triploid production

While early attempts at producing triploids had varying success (i.e. a variable proportion of the fish produced were still diploids) careful application of the pressure shock technique appears more reliable; Trevor Whyatt (Allenbrook Trout Farm) claims a very high success rate - close to 100%.

Any "failure" would result in normal diploid fish that would mature and be capable of spawning normally.

Availability and cost

All-female triploid brown trout are in commercial production at a number of fish farms. Much of the pioneering work has been undertaken by Trevor Whyatt at Allenbrook Trout Farm. He produces large numbers of eggs, which he sells at £13.50 per thousand compared to £12.50 for diploids. There is a slightly lower survival rate in triploid fry. These two factors

increase the cost of triploid production compared to diploid. Allenbrook charge £3.00 per lb for triploid stock fish compared to £2.75 for diploids. Ray Hill (Houghton Club) has a small premium on triploids. Westacre (Kings Lynn) charge £2.75 for both triploids and diploids, absorbing the extra cost. Croxley Hall Trout Farm (Rickmansworth) supply only triploids. Other possible suppliers are Anna Valley Fish Farm, Andover; Rooksbury Mill Fishery, Andover, (mixed-sex triploids); Roadwater Fisheries; and Lechlade Trout Farm. Contact details are given at the end of this note.

Concern over 'GMOs'

With current concerns over GMOs ('genetically modified organisms') and chemicals used in food production all manipulations such as sex reversal (all-female stocks) and triploid production will be carefully scrutinised.

The production of all-female stocks involves hormone treatment of the "father" (a genetic female that is treated to develop into a functional male). However, there is no hormone treatment of the offspring (the fish of concern here) and the eggs are perfectly normal females.

Triploids are not GMOs in that there is no introduction of genetic material from other organisms. Although triploidy sometimes occurs naturally in fish (Benfey 1999), it can be deliberately produced, usually by high pressure shock at the appropriate development egg stage. Triploids have the same genetic material as diploid trout but more of it, three sets of chromosomes as opposed to two. A key concern about 'GMOs' is that their use may result in their foreign genes being inadvertently released. The whole point about the proposal to use triploids is they are infertile and will not release their genes into the wild. Any "failures" are entirely normal diploids.

A consideration of limitation and risks.

In the past it has been suggested that triploids are more aggressive than diploids and may displace them from the optimal territories. As all-female triploids do not mature and continue feeding while diploid fish are spawning it has been suggested that they may prey on the eggs of wild stocks. It has also been suggested that stocked triploids may lock-up a significant part of the ecological resource without contributing to future recruitment.

These issues are now considered in turn.

The origin of the suggestion that triploids may be more aggressive than diploids is obscure. There is no such evidence in scientific reviews, and user experience with brown trout in the UK is that the diploids are more aggressive. Further, the evidence that any growth advantage of triploids in captivity is lost when they are reared together with diploids suggests that triploids are not dominant.

The fact that triploids continue to feed through the autumn while maturing diploid fish do not points to the possibility that triploids could predate on the eggs of spawning fish - certainly immature trout are known to do so. However, this has not been observed, and Ray Hill states that the triploids remain in their natural territories and do not follow spawning fish onto the redds. Overall, however, there is insufficient evidence to answer this concern with authority.

The issue of locking up a significant part of the ecological resource without contributing to future recruitment is an interesting one. Preventing the fish from contributing to recruitment is of course a prime reason for the use of triploids. The extent to which they divert ecological resources depends upon stocking levels and the production of wild fish. It must be assumed that there is spare "ecological resource" to justify restocking in the first place and stocking must be at an appropriate level whether it involves diploids, triploids or rainbow trout. Trevor Whyatt suggests that if you over-stock with triploids it is they that are displaced downstream, not the wild fish.

The overwhelming argument in favour of use of triploids is that it will cause no permanent damage to the wild stock. If any undesirable effects become apparent merely stopping stocking will cause the problem to disappear.

It appears that the extent of use of triploid brown trout for restocking in rivers in Southern England is considerable but unknown. A survey of its extent is strongly recommended; this could be usefully combined with canvassing user views and experiences.

There remain some uncertainties regarding potential interactions with wild diploids, and over-winter survival. These could readily be addressed with some straightforward monitoring of experimental stockings. This would greatly help the case for more extensive use of triploids.

Conclusions

All-female triploids would appear to satisfy the requirements for a fish for stocking that have all the beneficial attributes of diploids without the genetic risk to the native stock. They appear to be in extensive use in the south of England at least, being preferred for their higher over-winter survival in both farm and river. Consideration of a firm policy for their use in situations where a genetic risk is perceived with the use of diploids would appear to be a sound and justified development. Before that is done, however, it would be prudent to examine in more detail the performance of stocked all-female triploids, especially with respect to possible interactions with wild fish.

References

Anon (1999) Minimising the interaction of cultured and wild fish: a comprehensive evaluation of the use of sterile, triploid, Atlantic salmon. Final report of EU AIR3-CT94-2216. Marine Laboratory, Aberdeen; Gatty Marine Laboratory, St Andrews; University College, Galway; Institute of Marine Research, Bergen. 35pp.

Benfey, T. J. (1999). The physiology and behaviour of triploid fishes. *Reviews in Fisheries Science*, 7(1) 39-67.

Crozier W.W. and Moffatt I.J.J. (1989) Experimental production of triploid brown trout, *Salmo trutta* L, using heat shock. *Aquaculture and Fisheries Management*, 20; 343-353.

Dillon, J.C., Schill, D.J. and Teuscher, D.M. (2000) Relative return to creel of triploid and diploid rainbow trout stocked into eighteen Idaho streams. *N. Am. J. Fish. Mgmt*, 20: 1-9.

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

Johnstone, R. T. H., Simpson, A. F., and Whitehead, C. (1979). Sex reversal in salmonid culture. Part 2, the progeny of sex-reversed rainbow trout. *Aquaculture* 18: 13-19.

Lincoln, R. (1996). Progress towards the commercial production of triploid brown trout. *Trout News*, 22, 23-28.

Lincoln, R and Bye, V. J. (1987). Growth rates of diploid and triploid rainbow trout (*Salmo gairdneri* R.) over the spawning season. Page 134 in D. W. Idler, L. W. Crim, and J. M. Walsh, editors. Proceedings of the third international symposium on the reproductive physiology of fish. Memorial University of Newfoundland, Memorial Press, St. John's.

Purdom, C. (1983) Genetic engineering by the manipulation of chromosomes. In: Genetics in Aquaculture (ed. By N.P. Wilkins and E.M. Gosling), pp287-300. Elsevier, Amsterdam.

Sheehan, R.J., Shasteen, S.P., Suresh, A.V., Kapuscinski, A.R. and Seeb, J.E. (1999) Better growth in all-female diploid and triploid rainbow trout. *Trans. Am. Fish. Soc.*, 128: 491-498.

Stillwell, E. J. and Benfey, T. J. (1997). The critical swimming velocity of diploid and triploid brook trout. *Journal of Fish Biology*, 51, 650-653.