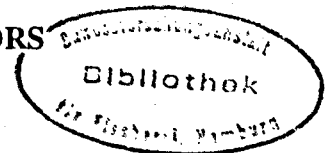


NOT TO BE CITED WITHOUT PRIOR REFERENCE TO AUTHORS



International Council for
the Exploration of the Sea

Theme Session on Anadromous and
Catadromous Fish Restoration Programmes:
A Time for Evaluation
CM 1996/T:4

EVALUATION OF A STOCKING PROGRAMME TO ENHANCE SPRING SALMON RUNS ON THE RIVER TEST, SOUTHERN ENGLAND

I.C. Russell, E.C.E. Potter, D.J. James
DFR Fisheries Laboratory
Pakefield Road
Lowestoft
Suffolk, NR33 0HT
England

and

I.K. Johnson
Environment Agency - Southern Region
Guildbourne House
Chatsworth Road
Worthing, West Sussex BN11 1LD
England

ABSTRACT

Catches of salmon, and spring running multi-sea-winter (MSW) fish in particular, have declined markedly on the River Test in southern England over the past thirty years. In the late 1980's, in an effort to arrest this decline, local fishery managers sanctioned the stocking of salmon parr and pre-smolts derived from a Scottish salmon stock characterised by a large proportion of MSW fish. More recently, the size of the stocking programme has been increased, with very large numbers of fry and parr being introduced. There were significant differences in allele frequencies at the *AAT-4** locus between fish from the River Test and from the introduced stocks and this could therefore be used as a marker to assess the effects of the stocking programme. Changes in the frequency of the *AAT-4*100* allele indicate that the stocking has resulted in a significant introgression of the Scottish genotype into the local stock. However, there has been no evidence of an increase in the MSW component over the duration of the enhancement programme. The possible reasons for this are discussed.

INTRODUCTION

The River Test is located in southern England. It rises in chalk downlands and flows southwards, collecting a number of spring-fed tributaries before discharging into Southampton Water and the English Channel. The river is approximately 60 km in length and has a relatively stable flow as a result of the high proportion of groundwater. A smaller river, the Itchen, runs adjacent to the Test and also discharges into Southampton Water. Both rivers have had a widespread reputation for providing high quality game fishing for both trout (*Salmo trutta* L.) and salmon (*Salmo salar* L.). However, there has been a marked decline in the catches of salmon in these rivers over the past thirty years (Russell *et al.*, 1995; NRA., 1993). The reasons for the decline are not precisely known, but a number of factors may have contributed, including: poor egg to alevin survival caused by siltation of spawning areas (Scott and Beaumont, in press), poor survival to smolt caused by low river flows and predation (NRA., 1993), and high exploitation (Anon., 1995). There has also been a progressive decline in the proportion of adult salmon returning to the river as multi-sea-winter (MSW) fish. The reasons for this are unclear, but similar reductions in the mean sea age at maturity have been reported for other salmon stocks in the region (Gough *et al.*, 1992) and in other parts of the North Atlantic (Anon., 1995).

The age at which salmon mature appears to be affected by environmental factors, possibly operating both in freshwater and the sea, and the genetic make-up of the fish (Saunders *et al.*, 1983; Piggins, 1974). The age composition of returning salmon may therefore be related to environmental changes affecting growth and maturation of individual fish or selective pressures operating on the phenotypic differences between fish having a genetically based tendency to mature at different ages. It has therefore been suggested that one way to enhance the MSW component of a salmon stock would be by stocking with juvenile salmon derived from a population which naturally produces a higher proportion of MSW fish (SAC, 1994).

This latter course of action was adopted by local managers on the River Test, as part of a larger stock enhancement scheme, in an attempt to halt the decline in the MSW component of the stock and to enhance runs generally. An enhancement programme was established in the late 1980s, and initially, eggs were introduced from a Scottish farmed-salmon stock, which produced a high proportion of MSW salmon.

Studies of polymorphic loci in the local Test stock and the introduced stock indicated a marked difference in allele frequencies at the enzyme aspartate aminotransferase (*AAT-4**) locus (Thompson & Russell, 1991; Hovey *et al.*, 1989; Youngson *et al.*, 1989). The frequency of the *AAT-4*100* allele could therefore be used as a marker to indicate changes in the genetic structure of the local salmon population following the stocking. Although this was not the subject of a specific study, samples collected for other purposes provide information on the change in the local stocks. This paper examines the evidence that there has been introgression of Scottish genotypes into the Test stock and considers the effect on the sea-age at maturity of the stock.

METHODS

The stocking programme on the River Test commenced in 1987. Initially, ova were obtained from two locations, a Scottish fish farm stock characterised by late (MSW) maturation and local Test fish. Details of the individual salmon selected as broodstock are not available. These fish were reared at hatcheries in the Test catchment and were stocked as parr or pre-smolts in batches of up to about 20K. The numbers of fish introduced to the River Test each year, together with the origin of each batch, are presented in Table 1.

Subsequently, efforts were made to greatly increase the scale of the enhancement programme (Table 1), and a broodstock of salmon of Test and Scottish origin was established at a commercial salmon farm in Scotland. In most years since 1991, ova derived from this source have been transported to various hatcheries for rearing-on and stocking. The number of fish stocked has increased greatly, with up to 711K fish released in a single year. However, due to constraints on available rearing space most of these fish have been released in the summer months as fry rather than parr.

Since 1993, parr have been reared from broodstock taken from fish returning to the Test. Fishermen, riparian interests and local fishery managers have collaborated in a 'catch and donate' policy, whereby fish caught by anglers have been transported to a large holding tank for later use as broodstock.

The performance of some batches of introduced hatchery-reared parr has been assessed by means of coded wire microtagging (Jefferts *et al.*, 1963). More recently microtags have also been applied to emigrating smolts; these fish have comprised juveniles derived from both natural spawning and unmarked, reared fish stocked into the river. The tag recovery data have been used to evaluate the effectiveness of the enhancement programme and to assess the levels of exploitation and marine survival of various tagged groups using the technique of run-reconstruction modelling (Potter & Dunkley, 1993). The run-reconstruction model also provides an estimate of the proportion of the one-sea-winter (1SW) recruits from each tagged group maturing as grilse (1SW fish) and the proportion remaining at sea for more than one year. This therefore provides a measure of any change in the mean age at maturation.

Livers, for electrophoretic analysis of the *AAT-4** locus, were collected from samples of the hatchery-reared parr stocked in most years from 1989 (Table 1). In 1989 and 1990 liver samples were also collected from parr caught at various sites in the River Test by electric fishing. In accordance with common practice for sampling salmon populations, efforts were made to ensure tissue samples were obtained from at least 50 individuals. An attempt was made to collect a further sample of parr from the Test in 1995, however, parr densities were very low and insufficient fish could be obtained from areas where stocking had not taken place. Parr originating from natural spawning in the River Itchen were sampled at this time.

Genetic variation at the *AAT-4** locus was identified by horizontal starch gel electrophoresis using a continuous amino-citrate buffer system, pH 6.1 (Clayton & Tretiak, 1972); staining was as described by Allendorf *et al.* (1977).

RESULTS

The frequency of the *AAT-4*100* allele for each of the batches of fish sampled are given in Table 2, together with: date of sampling, sample size, standard error and 95% confidence interval. The table also indicates whether the allele frequencies differ significantly from the expectations of the Hardy-Weinberg principle using a log likelihood χ^2 (G Test) (Ferguson, 1980). Parr taken from the wild in the River Test in 1989 and 1990 had *AAT-4*100* allele frequencies of 0.11 and 0.06. Samples of the parr reared from Scottish broodstock between 1989 and 1991 had *AAT-4*100* allele frequencies of 0.72 to 0.89. In all these samples the genotypic distribution in the samples complied with the expectations of the Hardy-Weinberg principle. A further sample of parr stocked in 1992 (Table 2) were derived from a different source (Test and Scottish parents) to those previously introduced and had an *AAT-4*100* allele frequency of 0.70, very similar to the other introduced fish. However, these fish did not comply with the expectations of the Hardy-Weinberg principle ($P < 0.01$).

In 1994 and 1995, parr were reared from adult fish returning to the River Test. The *AAT-4*100* allele frequencies for these fish were intermediate between the original Test stock and the Scottish stock, with values of 0.53 and 0.58. In these samples the genotypic distribution in the samples did not comply with the expectations of the Hardy-Weinberg principle ($P < 0.05$ and < 0.001 respectively). It was not possible to collect a sufficiently large sample of parr from the wild in the Test in 1995. However, a sample of naturally produced parr was obtained from the River Itchen, which has also been stocked with Scottish fish, although to a lesser extent. This sample had an *AAT-4*100* allele frequency of 0.58, which is very similar to that obtained from the Test hatchery samples in 1994 and 1995.

Table 3 gives the estimated numbers of microtagged fish recaptured (both 1SW and MSW fish) in the interception fisheries in Faroes (no tags were recovered in the West Greenland fishery), Ireland and UK (Northern Ireland), and in homewater rod fisheries. As numbers of tag recoveries are low, data have been reported for three tagged groups: the stocked Test origin hatchery parr (1988-89 smolt year classes), the introduced Scottish fish (1987-91 smolt year classes) and for the River Test smolts (1992-93). For both of the groups of introduced fish (Test and Scottish) the estimated number of tag recoveries in all fisheries is very low with tag recovery rates in all fisheries of only 0.1% and 0.3% respectively. By contrast, tag recovery rates for the smolts were much higher at 2.3%.

The relatively small numbers of tag recoveries, and the small proportion of tag recoveries from MSW salmon in particular, preclude detailed comparison between groups. However, applying the tag recovery data to the run-reconstruction model provides estimates of the proportion of the tagged groups maturing as 1SW fish (Table 3). The proportion of each group estimated to mature as 1SW fish varies from 84 to 90% and does not indicate any significant increase in the MSW component over the period of stocking.

DISCUSSION

Variations in allozyme frequencies can be used to indicate genetic differences between populations of salmon from different areas (Ståhl, 1987). The greatest differences in allele frequencies among salmon populations in England and Wales have been observed at the *AAT-4** locus. Genetic differences indicated by electrophoretic analysis are generally thought to be selectively neutral, in that slight variations in the proteins produced by different alleles are unlikely to affect the outcome of fishes' lives. They may therefore be used as markers to indicate introgression of genetic material from the introduced stock into the native river population.

The allele frequencies at the *AAT-4** locus for the Scottish origin parr introduced into the River Test were markedly different from the native Test stock at the outset of the study. The *AAT-4*100* allele frequencies found in the River Test parr in 1989 and 1990 were similar to those reported for salmon populations in three neighbouring chalk-stream catchments, the Rivers Itchen (0.02-0.18 in 1988-89), River Frome (0.10 in 1995) and River Piddle (0.15 in 1995) (Hovey *et al.*, 1989; I C Russell, unpublished data). The allele frequencies for the Scottish parr were similar to those reported for other Scottish farmed (0.70 - 0.97) and wild (0.75 - 0.85) stocks (Youngson *et al.*, 1989). Although insufficient samples were taken to follow the progress of the genetic introgression into the Test stock, it is evident from the samples taken in 1994 and 1995 that there had been a marked change in the genetic composition of the spawning stock. The frequency of the *AAT-4*100* allele in parr derived from spawners taken from the wild had increased to intermediate levels of 0.53 and 0.58 in the two years respectively. This indicates that the introduced stock were contributing to the adult population, but not that they had necessarily spawned successfully with the River Test fish in the wild.

It proved impossible to collect a sufficient sample of parr derived from natural spawning in the river Test in 1995 because of the continuing poor wild production and the difficulties of distinguishing naturally spawned fish from those stocked as fry. However, the sample of naturally produced parr obtained from the River Itchen, had an *AAT-4*100* allele frequency of 0.58, very similar to that obtained from the Test hatchery samples. This therefore indicates that the Scottish genotype had been incorporated into this stock. However, the genotypic frequencies differed significantly from the expected Hardy-Weinberg ratios. It is possible that this is the result of selection operating on different genotypes, but, it is more likely that it reflects non-random mating. This may be because the parr are derived from mating between fairly distinct parental groups. Alternatively, it is possible that as a result of the relatively low population densities in the river the sample was derived from few families.

Overall, the return rates from the stocking programme on the River Test have been disappointingly low. Nevertheless, the scale of the stocking programme has been sufficient to result in the stocked fish making a significant contribution to the returning adult runs. Although it appears that the genetic composition of the Test stock has been altered by the introduction of Scottish fish, there is no evidence from microtag recoveries or the returns to the river that there has been any increase in the

proportion of MSW fish. This may be because, although the Scottish stock from which the broodstock were taken was known to produce a high proportion of MSW salmon the individual fish selected did not possess strong MSW characteristics. It is also possible that the fish failed to demonstrate the MSW phenotype because of differences in the environmental conditions experienced. This would be consistent with the finding of Saunders *et al* (1983) who divided a group of hatchery-reared salmon into two batches; one was transferred to a sea cage and the other released into a river. While only 1% of the fish in the cage matured as 1SW fish, 63% of the adults returning from the released batch did so after one sea-winter. This suggests that environmental factors can have a strong overriding influence over a genetic trait to mature at a particular age.

This case study emphasises the difficulty of selectively enhancing particular components of a stock. The use of fish derived from MSW parents confers no guarantee that MSW offspring will subsequently be produced both because of the difficulties of knowing whether the eggs contain MSW genotypes and because of the strong environmental influences on maturation.

REFERENCES

- Allendorf, F.W., Mitchell, N., Ryman, N. & Ståhl, G. (1977). Isozyme loci in brown trout (*Salmo trutta* L.); detection and interpretation from population data. *Hereditas*, 86, 179-190.
- Anon. (1995). Report of the ICES North Atlantic Salmon Working Group. ICES, CM 1995 / Assess 14, Ref:M.
- Clayton, J.W. & Tretiak, D.N. (1972). Amino-citrate buffers for pH control in starch gel electrophoresis. *J. Fish Res. Bd. Can.*, 29, 1169-1172.
- Ferguson, A. (1980). *Biochemical Systematics and Evolution*. Glasgow: Blackie.
- Gough, P.J., Winstone, A.J. & Hilder, P.G. (1992). A review of factors affecting the abundance and catch of spring salmon from the River Wye and elsewhere, and proposals for stock maintenance and enhancement. NRA Publication, Welsh Region.
- Hovey, S.J., King D.P.F., Thompson, D. & Scott, A. (1989). Mitochondrial DNA and allozyme analysis of Atlantic salmon (*Salmo salar* L.) in England and Wales. *J. Fish Biol.*, 35 (Suppl. A), 253-260.
- Jefferts, K.B., Bergman, P.K. & Fiscus, H.F. (1963). A coded wire tagging system for macro-organisms. *Nature*, 198, 460-462.
- NRA. (1993). River Test Catchment Management Plan Final Report. National Rivers Authority Southern Region, HMSO, July 1993.
- Piggins, D.J. (1974). The results of selective breeding from known salmon and grilse parents. Salmon Research Trust for Ireland, Annual Report XVIII, pp 35-39.

Potter, E.C.E. & Dunkley, D.A. (1993). Evaluation of marine exploitation of salmon in Europe. In: (Ed. D.H. Mills) *Salmon in the Sea and New Enhancement Strategies*, Fishing News Books, Oxford, pp 203-219.

Saunders, R.L., Henderson, E.B., Glebe, D.B. and Loudenslager, E.J. (1983). Evidence of a major environmental component in the determination of grilse : larger salmon rates in Atlantic salmon (*Salmo salar*). *Aquaculture*, **33**, 107-118.

Russell, I.C., Ives, M.J., Potter, E.C.E., Buckley, A.A. & Duckett, L. (1995). Salmon and migratory trout statistics for England and Wales, 1951-1990. Fisheries Research Data Report No 38., Directorate of Fisheries Research, Lowestoft, 252 pp.

Salmon Advisory Committee (1994). Run timing of Salmon. MAFF publications, London, 55 pp.

Scott, A. & Beaumont, W.R.C. (in press). Improving the survival rates of Atlantic Salmon (*Salmo salar* L.) embryos in a chalkstream. Proc. Inst. Fish. Mgmt. Annual Study Course, University of Wales, Cardiff, 14-16 September 1993.

Ståhl, G. (1987). Genetic population structure of Atlantic salmon. In: N. Ryman and F. Utter (Editors), *Population Genetics and Fishery Management*, University of Washington Press, Seattle, WA, pp 121-140.

Thompson, D. & Russell, I.C. (1991). Allele frequency variation at the *AAT-4* locus as a potential measure of the relative performance of native and introduced Atlantic salmon in the River Test. *Aquaculture*, **98**, 243-247.

Youngson, A.F., Martin, S.A.M., Jordan, W.C. & Verspoor, E. (1989). Genetic protein variation in farmed Atlantic salmon in Scotland. Comparison of farmed strains with their wild source populations. Department of Agriculture and Fisheries for Scotland, Scot. Fish Res. Rep., **42**, 12 pp.

Table 1. Numbers of salmon stocked and tagged in the River Test, 1987-95.

Origin of fish #	Release date	Stage	No. of fish introduced	No. tagged
Scottish Test	Apr-87	1+parr	10,504	10,504
Scottish Test	Apr-88	1+parr	6,023	6,023
Scottish Test	Apr-88	1+parr	1,438	1,438
Scottish - Spey Test	Apr-89	1+parr	20,598	20,598
Scottish - Spey Test	Apr-89	1+parr	15,211	15,211
Scottish - Spey Test	Mar-90	1+parr	17,475	17,475
Scottish Test/Scottish Test	Mar-91	1+parr	10,817	10,817
Test/Scottish Test	Aug-91	0+parr	710,817	17,724
Test/Scottish Test	Feb-92	1+parr		17,928
Test/Scottish Test	Aug-92	0+parr	661,928	22,686
Test/Scottish Test	Jul-93	0+parr	207,628	21,719
Test	Oct-94	0+parr	38,806	38,806
Test	Jul-95	0+parr	40,751	33,251
Test/Scottish	Jul-95	0+parr	91,100	-

See text for details.

Table 2. Allele frequencies at the sAAT-4* locus for introduced and River Test origin fish.

Category	Stage	Date sampled	Sample size	Allele frequency			Hardy-Weinberg probability *
				sAAT-4*	se	95% C.L.	
Original Test stock	0+ parr	Oct-89	55	0.11	0.030	0.058	N.S.
Original Test stock	0+ parr	Oct-90	45	0.06	0.025	0.049	N.S.
Introduced stock	1+ parr	Jan-89	57	0.72	0.042	0.082	N.S.
Introduced stock	1+ parr	Mar-90	30	0.83	0.048	0.095	N.S.
Introduced stock	1+ parr	Jan-91	50	0.89	0.031	0.061	N.S.
Introduced stock	0+ parr	Aug-92	60	0.70	0.042	0.082	<0.01
Current Test stock	0+ parr	Oct-94	60	0.53	0.046	0.089	<0.05
Current Test stock	0+ parr	Jul-95	50	0.58	0.049	0.097	<0.001

* Represents probability of variation from Hardy-Weinberg expectation.

Table 3. Estimated tag recoveries by sea-age class and proportion of fish maturing as MSW salmon.

Smolt years	Origin	Number tagged	Est. 1SW Recoveies			Est. MSW Recoveies			Tag recovery rate (%)	Est. % 1SW (from RR model)
			Local rod	Ire/NI	Faroe	Local rod	Ire/NI	Faroe		
1988-89	Original Test (hatchery parr)	21,234	7	17.1		1			0.11	90
1987-91	Introduced stock (Scottish)	60,832	62	73.8		8	12	2.3	0.26	86
1992-93	Recent Test ('wild' smolts)	3,931	50	28.9	1	10			2.29	84