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Stocking success of Scottish Atlantic salmon in two Spanish rivers

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A previous analysis of proportions of stocked and wild Atlantic salmon among angled fish in the Rivers Asón and Nansa in northern Spain based on the analysis of *MEP-2** genotypes is extended. The results reinforce the initial conclusion that returns of stocked Scottish salmon are significantly lower than returns for wild fish.

Key words: malic enzyme; *MEP-2**; *Salmo salar*; introductions.

The biological consequences of introductions of non-native Atlantic salmon *Salmo salar* L., for wild populations of conspecifics are poorly understood (Verspoor, 1989). Circumstantial evidence suggests that the consequences may be negative (Hindar *et al.*, 1991). The long-term genetic impact of introductions of non-natives will be largely dependent on the numbers of foreign fish which survive to reproduce. In a previous study (Garcia de Leániz *et al.*, 1989), evidence was found of a lower return rate of stocked Scottish salmon to the angling fisheries of the Rivers Asón and Nansa, in northern Spain, compared to wild fish. The cause appeared to be poorer survival of the introduced fish rather than differences in behaviour affecting availability to anglers. Here the initial analysis of the relative return rates was extended to include adult fish caught by angling in 1989 and 1990, as well as data on wild fry, parr and smolts from the 1986, 1987 and 1988 year classes sampled in 1988. The new analysis covers the year classes 1984–1988 in the Asón and 1984–1986 in the Nansa.

The stocked components of the 1984–1988 year classes in the two rivers derive from imported ova obtained, with two exceptions, from the Polly Estates hatchery in Scotland. The exceptions relate to the Asón where for the 1984 year class 56% of salmon stocked derived from Polly Estates and 44% from a farmed Icelandic source, and for the 1987 year class where 34% of stocked fish were from Polly Estates, 34% from the River Spey in Scotland, 5% from the River Shin in Scotland, and 27% from Silver Cup, a farm strain of Norwegian origin from Denmark. The stocking was carried out at the eyed ova stage and the relative expected proportions of introduced and wild ova were estimated from redd counts (Garcia de Leániz *et al.*, unpublished). Fish were typed for variation at the diallelic *MEP-2** locus (E.C. 1.1.1.40—previously called *Me-2*) using starch gel electrophoresis (Verspoor, 1988). As previously (Garcia de Leániz *et al.*, 1989), estimates of the proportion of stocked and native fish were derived from the extent of the deficit in the proportion of heterozygote genotypes (Mork *et al.*, 1984) based on the Wahlund effect (Hartl & Clark, 1989). Departures from C-H-W genotype proportions were expressed in terms of F_{IS} (1-observed/expected heterozygotes) and assessed for significance using the *G*-test adjusted by Williams' correction (Sokal & Rohlf, 1981). Heterozygote deficiencies were expected for physical mixtures of stocked northern European and wild Spanish salmon given that the former generally (Verspoor & Jordan,

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TABLE I. Frequencies of *MEP-2** genotypes observed among juvenile fish sampled in April 1988 in the Rivers Asón and Nansa

River stage/age	No. typed	Year class	110/100	100/125	125/125	F_{IS}	*G*-test probability	Frequency **100*
River Asón								
Fry 0+	14	1988	13	1	0	+0·37	NS	0·964
Parr 2+	8	1986	7	1	0	−0·07	NS	0·938
1+	33	1987	16	8	9	+0·49	0·005	0·606
	1	N/A	0	1	0	−1·0	NS	0·500
	42	Total	23	10	9	+0·46	0·003	0·667
Smolts 2+	5	1986	4	0	1	+1·0	NS	0·800
1+	23	1987	9	6	8	+0·48	0·023	0·522
	1	N/A	1	0	0	0	NS	1·00
	29	Total	14	6	9	+0·57	0·002	0·586
River Nansa								
Fry 0+	9	1988	9	0	0	0	NS	1·00
Parr 1+	2	1987	1	1	0	−0·20	NS	0·750
Smolts 2+	1	1986	1	0	0	0	NS	1·00
1+	24	1987	18	4	2	+0·41	0·06	0·833
	25	Total	19	4	2	+0·40	0·08	0·840

1989), and the planted stocks specifically (Verspoor *et al.*, unpublished), are characterized by *MEP-2*100* frequencies of <0·500 while among wild Spanish salmon they range from 0·85 to 1·0 (Verspoor *et al.*, unpublished).

Significant heterogeneity in genotype frequencies was found among juvenile fish (Table I). The 1986 and 1987 year classes of parr from the Asón were different (G=4·2, d.f.=1, P=0·04) but not the smolts (G=2·6, d.f.=1, P=0·11). No significant differences occurred between parr and smolts from the same year class in the Asón but there was significant heterogeneity among juvenile year classes (G=17·4, d.f.=4, P=0·0016). Among Nansa juveniles, the only significant difference was between the 1987 (parr and smolts) and 1988 (fry) year classes (G=3·7, d.f.=1, P=0·05). The 1987 Asón parr were significantly different from the 1987 returning adults (Table II; G=9·4, d.f.=2, P=0·009) and from the 1988 wild fry, unaffected by stocking (G=6·2, d.f.=1, P=0·013). No comparison of juveniles and adults of the same year class was possible for the Nansa.

For adult fish, the extended analysis (Table II) shows year class heterogeneity in the Asón (G=11·5, d.f.=4, P=0·02—the 1983 year class excluded), attributable to the 1988 year class being significantly different from the others (G=8·4, d.f.=1, P=0·0038). Among the other year classes there is no evidence of heterogeneity (G=2·7, d.f.=3, P=0·43). No year class heterogeneity was detected among the angled Nansa adults (G=3·7, d.f.=2, P=0·14—the 1987 year class excluded), and genotype frequencies among angled fish were not significantly different from post spawning kelts (Table II; G=2·25, d.f.=1, P=0·13). Thus, fish caught by angling are likely to be representative of the adult population as a whole.

Heterozygote deficiencies were observed among the 1987 Asón parr and smolts, the 1985 year class of adults from the River Asón, 1987 Nansa smolts, and 1986 Nansa adults (Tables I and II) though only for the Asón samples were they significant. Expected proportions of stocked and wild fish estimated from the heterozygote deficits in the adult year classes were one-third and one-quarter of the numbers expected based on numbers of ova stocked and estimates of natural egg deposition (Table III). These were calculated using the *MEP-2*100* frequency of 0·337 found for the 1986 year class in the River Polly (Jordan *et al.*, 1992), the source of the brood stock used to generate the eggs stocked in the Asón and Nansa in 1986. The estimated proportions of stocked fish among juveniles were higher than expected in the River Asón and lower than expected in the Nansa. The expected numbers of stocked fish among sampled adults in the different year classes differed in three of the five year classes of adults in the Asón and in one of the three year

TABLE II. Frequencies of *MEP-2** genotypes observed among adult fish sampled in 1988–1990 rod and line fisheries in the Rivers Asón and Nansa

Year class	110/ 100	100/ 125	125/ 125	Total	F_{IS}	*G*-test probability	Frequency **100*
River Asón (number caught = 424, number typed = 282)							
1983	1	0	0	1	0	—	1·000
1984	17	3	0	20	− 0·08	NS	0·925
1985	184	20	4	208	+ 0·234	0·008	0·933
1986	23	1	0	24	− 0·02	NS	0·979
1987	11	3	0	14	− 0·12	NS	0·893
1988	4	4	1	9	+ 0·00	NS	0·667
N/A	6	0	0	6	0	NS	1·000
Total	246	31	5	282	+ 0·184	0·011	0·927
River Nansa (number caught = 91, number typed = 73)							
1984	10	3	0	13	− 0·13	NS	0·885
1985	34	1	0	35	− 0·01	NS	0·986
1986	19	2	1	22	+ 0·45	0·111	0·909
1987	2	0	0	2	0	NS	1·000
N/A	1	0	0	1	0	NS	1·000
Total	66	6	1	73	+ 0·21	0·180	0·945
River Nansa (Kelts; number typed = 31)							
Kelts	25	6	0	31	− 0·11	NS	0·903

TABLE III. Estimated proportions of stocked and native fish based on stocking levels relative to estimates of natural egg deposition and observed genotype proportions in year classes showing herterozygote deficiencies indicative of population mixing

Group	Estimated proportions of fish (%)		Estimated *MEP-2** genotype proportions			*n*	Estimated *MEP-2*100*
	From ova deposition	From genotypes	100/ 100	100/ 125	125/ 125		
River Asón—1985 adults							
Stocked	17	4	0·9	3·7	3·6	8·2	0·337
Wild	83	96	183·1	16·3	0·4	199·8	0·957
River Nansa—1986 adults							
Stocked	32	10	0·3	1·0	1·0	2·3	0·337
Wild	68	90	18·8	1·0	0	19·8	0·974
River Asón—1986 parr + smolts							
Stocked	11	25	0·3	1·0	1·0	2·3	0·337
Wild	89	75	6·7	0	0	6·7	1·00
River Asón—1987 parr + smolts							
Stocked	50	69	4·4	17·2	16·9	38·5	0·337
Wild	50	31	17·5	0	0	17·5	1·00
River Nansa—1987 parr + smolts							
Stocked	27	16	0·5	1·9	1·9	4·3	0·337
Wild	73	84	18·5	3·1	0·1	21·7	0·936

classes in the Nansa (Table IV). One further year class in the Nansa approaches significance. Across year classes the differences among adults were highly significant for both the Asón ($P<1\times10^{-8}$) and the Nansa ($P=2\times10^{-7}$) as were the differences in the expected proportions in the two rivers ($P=4\times10^{-4}$). However, the estimated observed proportions in the two rivers were not ($P=0{\cdot}57$).

The accuracy of the estimates of proportions of stocked and wild fish in samples will be influenced by sampling error and errors associated with the value of the frequency of

TABLE IV. Comparisons of expected and estimated numbers of stocked and wild fish among angled adults by year class for the Rivers Asón and Nansa

River/ year class	Expected proportion of stocked fish	Sampled adults				Fisher's exact test
		Expected numbers		Estimated numbers		
		Wild	Stocked	Wild	stocked	
River Asón						
1984	0·47	11	9	20	0	6×10^{-4}
1985	0·17	173	35	200	8	8×10^{-6}
1986	0·11	21	3	24	0	0·11
1987	0·50	7	7	7	0	3×10^{-3}
1988	0·12	8	1	9	0	0·5
River Nansa						
1984	0·26	10	3	13	0	0·11
1985	0·59	10	15	25	0	1×10^{-6}
1986	0·32	15	7	20	2	0·07

the **100* allele among stocked fish used to estimate mixing proportions. With the exception of 1985 and 1986, the sample sizes for year classes of angled adults were small, such that sampling error could be important. Sampling error could, for example, account for the deviation of genotype proportions among the 1988 adults in the Asón from the other year classes. Given that the stocking level was low relative to natural egg deposition in 1988 when compared to other years and natural levels of spawning (based on redd counts) were high, the high incidence of heterozygotes is unlikely to be due to a high level of stocked fish amongst which heterozygotes would be more common. Otherwise the consistent results across year classes indicate that the basic conclusion, that returns of stocked fish are significantly lower than those of wild fish, is not an artifact of sampling error. The basic conclusion stands if the assumed *MEP-2*100* frequency for stocked fish used to derive estimates of proportions of stocked and wild fish in the two adult year classes which showed heterozygote deficits, is only approximately correct. Though in both cases the stocked component was of Polly Estates origin, it is known that hatchery stock can deviate significantly from wild source populations (Verspoor, 1988). If the frequency was overestimated, the conclusion would be even more strongly supported. On the other hand, if it was as high as 0·5, the estimated numbers of stocked fish returning would still be less than half of that expected and the difference significant.

The estimate of the relative survival of the non-native fish is unlikely to be substantively influenced by any selective effect on *MEP-2** (Verspoor & Jordan, 1989). This is possible, given the differences in genotype frequencies among stocked and wild fish. Available evidence suggests that selection operates through differential growth and maturation (Jordan & Youngson, 1991; Jordan *et al.*, 1990) and thus will affect reproductive success primarily. However, even if associated with differential survival, selection coefficients are likely to be trivial compared to selective differences associated with the overall genetic differences between the non-native and native stocks.

The estimated proportions of stocked fish in the catches in the two rivers are not significantly different though, based on stocking levels, they are expected to be significantly higher among Nansa adults than among adults in the Asón. This suggests that stocked fish do less well in the Nansa than in the Asón. This may be the case. In the Nansa, an impassible dam confines salmon to the lower 7 km of the system and production of salmon in the system approaches carrying capacity. This is not true for the Asón which has 35 km of river accessible to spawning and appears to be far off its potential carrying capacity. Consistent with this, estimated proportions of stocked fish among juveniles (Table III) are lower than expected in the Nansa, though not significantly ($P=0{\cdot}25$). However, proportions in the Asón are significantly higher ($P=0{\cdot}027$) and more consistent with the differences between the two rivers being due to non-random sampling of the Asón, even though the Asón juveniles were collected at five different locations. Whether the differential performance of stocked and wild salmon is associated with the freshwater or marine phase of the life cycle is unclear. Either could be involved given that the fish were planted out as eyed ova. While the sampling of juvenile parr and smolts shows clearly that stocked fish do survive to the smolt stage, the data are inadequate to address the question of differential survival during the freshwater phase of the life cycle, particularly if the juvenile samples are unrepresentative of each river as a whole. As it stands, the observed heterogeneity in proportions of stocked fish could reflect spatial or temporal variation either in stocking levels or in the survival of stocked fish. Thus, while it is possible to conclude from the extended analysis that overall survival from egg to adult of the stocked fish is significantly lower, the analysis does not allow us to conclude why.

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