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S E Hartley and A D Pickering

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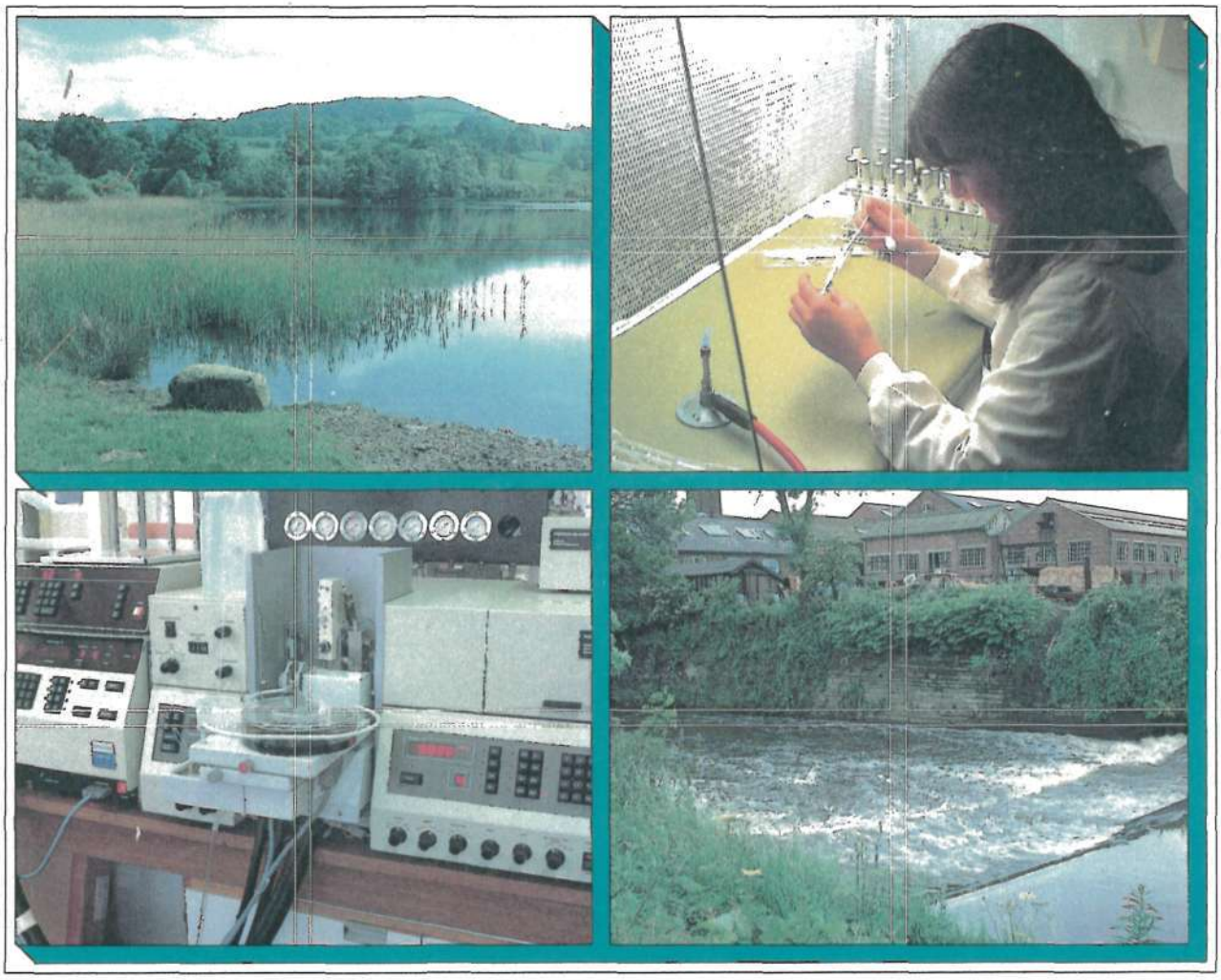

Natural Environment Research Council

Institute of Freshwater Ecology

Windermere Laboratory
Far Sawrey
Ambleside
Cumbria LA22 0LP

Telephone 05394 42468
Facsimile 05394 46914
Telex 94070416 WIND G

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S E Hartley* and A D Pickering

Project Leader:	A D Pickering
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* Department of Biological & Molecular Sciences, Stirling University, Stirling, Scotland FK9 4LA

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CONTENTS

Page

SUMMARY

Genetic analysis, using single locus probes for genomic DNA, revealed that the juvenile Atlantic salmon populations in the Rivers Leven, Rothay and Troutbeck were related but genetically distinct. This genetic differentiation is greater than might be expected (by comparison with other salmon populations in the UK) and it is recommended that no action is taken which might promote genetic exchange between the three rivers. Thus, future fisheries management practices should treat the salmon from each site as separate genetic stocks. It is unlikely that any attempts to encourage fish currently spawning in the River Leven (downstream of Windermere) to utilize the upper catchment will be successful.

The faster growth rate of juvenile salmon in the River Leven, compared with the River Rothay, probably results from a difference in temperature between the inflowing streams and the main outflow of Windermere. Precocious sexual maturation of some male parr was found in all three populations but the incidence (13-33%) is well within the range reported for other waters. Because of their enhanced growth rate, it is likely that some of the precocious males in the River Leven were 0+ fish.

A very high incidence of hybridization (>18%) between Atlantic salmon and brown/sea trout was found in Troutbeck but not in the other rivers. Mitochondrial DNA analysis of these hybrids revealed them to be the product of several, independent cross-fertilizations involving both sexes of both species. The implications of this finding are discussed in relation to the availability of suitable spawning sites in Troutbeck.

1. INTRODUCTION

1.1 Windermere salmon stocks

A recent NRA report (McCubbing, 1993) has shown that estimates of the populations of juvenile Atlantic salmon, *Salmo salar,* in the Windermere catchment are consistent with the estimates of numbers of returning adults. Highest densities of salmon fry and parr are found in the lower reaches of the River Rothay, between Rydal Water and Windermere, with the lower River Brathay, Troutbeck and the River Leven also acting as important nursery areas (Fig. 1). In the River Leven, salmon spawning activity is greatest in the first kilometre downstream of Newby Bridge Weir. The reason for such a concentrated spawning activity in this region of the River Leven is not clear but concern was expressed that intraspecific competition may be having a detrimental effect on the salmon populations in this area. It was also suggested (McCubbing, 1993) that an investigation be undertaken to ascertain the effect on upstream migration of adult salmon of the weir and fish pass at Newby Bridge and of the increased salmon rod fishery exploitation rate (post 1986) on the lake outlet channel upstream of the weir and fish-pass. The direct effects of such an exploitation might explain why some parts of the upper catchment appear not to be at their carrying capacity for juvenile salmon.

Implicit in the above approach is the presumption that, should such constraints to upstream migration be ameliorated, an increased number of spawning salmon would migrate into the upper catchment, perhaps reducing the apparent over-exploitation of spawning sites below Newby Bridge. Such a natural dispersion of salmon from the lower to the upper catchment is only likely to occur if the stocks of salmon at the various spawning and nursery sites within the Windermere catchment are components of a single, genetic population.

1.2 Genetic variability in Atlantic salmon

Modern molecular techniques have enabled the identification of discrete Atlantic salmon stocks. There is a major discontinuity between Atlantic salmon from mainland Canada and salmon from Europe. The European salmon can be further divided into populations inhabiting rivers that drain into the Baltic and those whose rivers feed into the eastern Atlantic Ocean (see Ståhl, 1987 for details). Resident (land-locked) and migratory Atlantic salmon can also co-exist as reproductively isolated populations within the same water body (Vespoor and Cole, 1989). Moreover, there is now genetic evidence for population differentiation of migratory Atlantic salmon both within and between river systems (King *et al*, 1993). Thus, as with many species of salmonids, the Atlantic salmon exhibits a remarkable degree of genotypic plasticity.

The main objective of the present study was to apply modern molecular techniques to the study of the genetics of juvenile Atlantic salmon from three different nursery areas within the Windermere catchment in order to assess whether genetically-distinct subpopulations exist. Although both allozyme and mitochondrial DNA analyses of Atlantic salmon in England and Wales provide evidence for population differentiation within and between river systems (Hovey *et al*, 1989; King *et al*, 1993), the amount of genetic variability detected by these methods is low. DNA profiling techniques with either multilocus or single locus minisatellite probes can provide sets of useful nuclear DNA markers for population genetic analysis where there is little detectable variability with protein electrophoresis. In 1990 Taggart and Ferguson described hypervariable minisatellite DNA single locus probes useful for population genetic analysis. Three single locus probes derived from Atlantic salmon and brown trout, which detect between 9 and 20 alleles ranging in size from 1.9 - 10.2 kilobase pairs (kbp) (Table 1), have been used in this study of Atlantic salmon populations in the Windermere catchment.

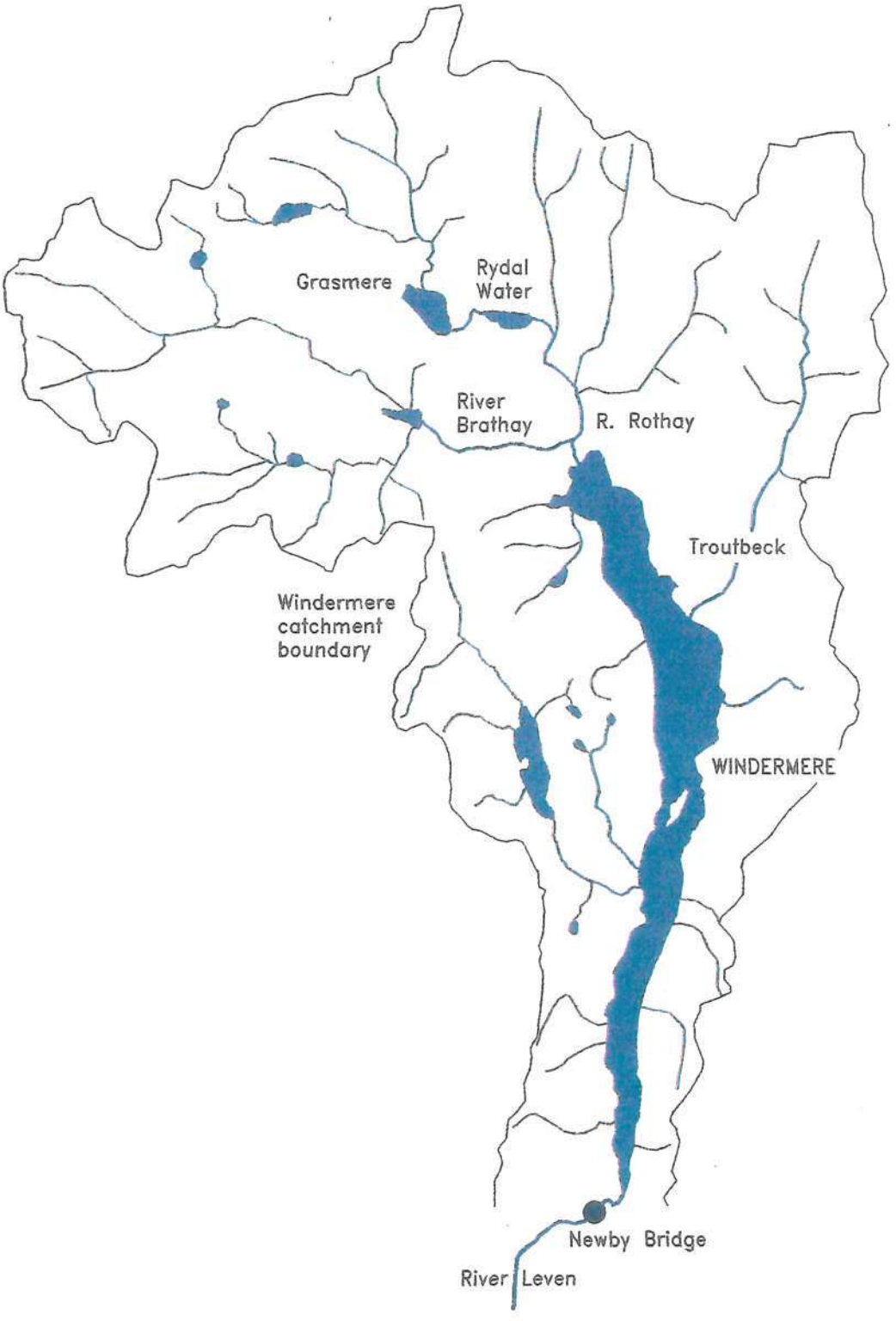

Fig.1 The Windermere Catchment

2. MATERIALS AND METHODS

2.1 Fish sampling

Juvenile Atlantic salmon were obtained by NRA fisheries staff using electrofishing techniques on the River Leven, Troutbeck and the lower River Rothay on 27 10 93 and delivered, alive, to the Windermere Laboratory of the Institute of Freshwater Ecology. An additional sample from Troutbeck was also obtained on 28 10 93. In total, 55 fish were taken from Troutbeck (fish numbers 1-19, 225-260), 99 from the River Leven (fish numbers 20-118) and 106 from the River Rothay (fish numbers 119-224). On receipt, the fish were anaesthetized in phenoxyethanol (1:2000), weighed and measured (fork length) and then individually deep-frozen at -80°C. Subsequently (04 11 93), the fish were transferred to Stirling University for DNA analysis.

2.2 DNA isolation and electrophoresis

DNA was isolated from livers using the technique described by Taggart *et al* (1992) for rapid isolation from large sample sizes. Approximately 4µg of DNA was digested overnight at 37°C with 20 units of the restriction enzyme *Hae*III. Electrophoresis through 25cm long 0.75% agarose gels in 0.5x Tris-phosphate (TPE) buffer (10x TPE: 108g Tris base, 15.5ml 85% phosphoric acid, 40ml 0.5M EDTA pH8.0/litre) was carried out at 47 volts for 24 hrs. An internal marker comprising the 1 kilobase reference ladder from Gibco-BRL and ΦX174 DNA fragments (Taggart and Ferguson, 1994) was included in every lane. Following electrophoresis, gels were capillary blotted to Hybond N nylon membrane (Amersham) using 0.5M NaCl/0.25M NaOH as the transfer buffer, having first been denatured for 1 h in 0.5M NaCl/0.5M NaOH.

2.3 DNA hybridization

Probe DNAs (see Table 1) were labelled with ^{32}P by random priming (Feinberg and Vogelstein, 1983) using a kit from Gibco-BRL.

Prehybridizations (4-5 hrs) and hybridizations (overnight) were carried out at 65°C in 3x SSPE pH7.7, 0.5% Marvel, 1% SDS, 6% PEG 8000 (20x SSPE pH7.7 = 210.2g NaC1, 31.2g $NaH_2PO_4.2H_2O$, 40ml 0.5M EDTA pH 8.0/litre). Sonicated salmon DNA (Sigma) was included as competitor during hybridizations. Post-hybridization washes, also at 65°C, were 30 min in 2x SSC / 0.1% SDS, followed by 2 x 30 min in 0.15-0.4x SSC / 0.1% SDS (20xSSC=175.3g NaCl, 88.2g Na_3 citrate/litre). Autoradiography was carried out at -70°C with intensifying screens.

2.4 Data analysis

The positions of all the alleles were transferred to acetate sheets which were than overlain on the autoradiographs of the marker system. This allows accurate typing of alleles taking into account any concentration effects within the lanes and any differential mobility across the gel. Genetic analysis was carried out using the BIOSYS-1 program of Swofford and Selander (1981).

Table 1: Details of probe DNAs and hybridization conditions

Probe	No of alleles detected[1]	Competitor DNA[2]	Final Wash
pSsa-45/1 (Atlantic salmon)	6(A-F) (1.9-3kbp)	45µg	0.4xSSC
pSsa-45/2 (Atlantic salmon)	17(A-Q) (4.6-10.2kbp)	134µg	0.15xSSC
pStr-9 (Brown trout)	9(A-I) (2.5-6kbp)	35µg	0.4xSSC

1 Number of alleles and thesize range in kilobase pairs (kbp) detected in a study of Atlantic salmon from the River Dee system, Aberdeenshire. n all cases A is the largest allele (Dr J B Taggart, personal communication).

2 The amount of sonicated salmon DNA (Sigma) included in the overnight hybridization per 200cm^2 of filter.

3. RESULTS

3.1 Fish

Of 260 fish examined, 53 were identified as mature male parr (18 from Troutbeck [32.7%], 13 from the River Leven [13.1%] and 22 from the River Rothay [20.75%]). The genotypes at all three loci were ascertained for 233 salmon (45 from Troutbeck, 86 from the River Leven and 102 from the River Rothay) (Appendix 1). Two of the three probes used (pSsa-45/1 and pStr-9) distinguish brown trout from Atlantic salmon. Three fish from the River Leven sample were found to be brown trout; 1 fish from the River Leven sample (1.01%) and 10 fish from the Troutbeck sample (18.18%) were found to be Atlantic salmon x brown trout hybrids (see later section).

3.2 Length distribution

Fish from the River Leven exhibited a bimodal length distribution with mean lengths of 9.7 and 14.9cm for the lower and upper modes, respectively (Fig. 2). A bimodal distribution was also seen in the fish from the River Rothay but in this case the mean lengths were 6.6cm and 10.6cm. Fish from Troutbeck exhibited a unimodal distribution around a mean of 11.5cm. The occurence of mature male parr was evenly distributed between both length modes in the River Leven fish but precocius males were only found in the upper mode in the fish from the River Rothay (Fig. 2). Individual details for each fish are presented in Appendix 1.

3.3 Observed genetic variation

The three probes used in this study detected between eight and fourteen alleles (Fig 3; Tables 2-4). A number of alleles detected in a study of the River Dee system in Aberdeenshire (Dr. J. B. Taggart, personal communication) were absent from the present study and a number of new alleles were found (Tables 2-4).

There was considerable genetic variability within each population at all three loci (Table 5). Genetic heterogeneity between the populations was characterized using a standard x^2 contingency table analysis (Table 6). **There is a marked and highly statistically significant genetic differentiation between the three populations.** The probability that such apparent differentiation is simply a chance effect and does not represent true genetic differentiation between the populations is less than 1 in 10,000. Analysis of allele frequencies shows that all three populations are in Hardy-Weinberg proportions at all three loci. A UPGMA dendrogram using Nei's (1978) unbiased genetic identity is shown in Fig. 4.

3.4 Comparison with another river system (River Dee, Scotland)

Allele frequencies at the same three loci (courtesy of Dr. J.B. Taggart) for distinct Atlantic salmon populations in two tributaries of the River Dee (the Girnock and the Baddagh Burns) were compared with those from the Windermere populations. The UPGMA dendrogram (Fig. 5) shows that the three Windermere populations are more distinct from one another than the two Dee populations are from one another.

3.5 Hybrids

Hybrids between Atlantic salmon and brown trout were detected in two populations. One fish in the Leven population (1.01%) and 10 fish in the Troutbeck population (18.18%) were hybrids. The direction of hybridization and the number of fish involved was determined by analysis of *Ava*II mitochondrial (mt) DNA genotypes and alleles at the Ssa-45/1 and Str-9 loci. Animal mt DNA is a small, rapidly-evolving, circular molecule which does not undergo recombination and is maternally inherited (Brown, 1985). The fragment patterns generated by the digestion of mt DNA by the restriction enzyme *Ava*II distinguish between Atlantic salmon and brown trout (Gyllensten and Wilson, 1987) and can also distinguish different brown trout individuals (Dr. R. A.

Hynes, personal communication). Total DNA from 2 Atlantic salmon, 3 brown trout and the 11 hybrids was digested with *Ava*II and probed with ^{32}P labelled brown trout mt DNA. The hybrid from the Leven population had an Atlantic salmon mother. The Troutbeck hybrids (Table 7) were the progeny of at least four crosses. One hybrid had an Atlantic salmon mother while the other 9 had brown trout mothers. Five different Atlantic salmon alleles and three *Ava*II genotypes in the mt DNA were observed indicating that the 9 hybrids with brown trout mothers were from at least three different families.

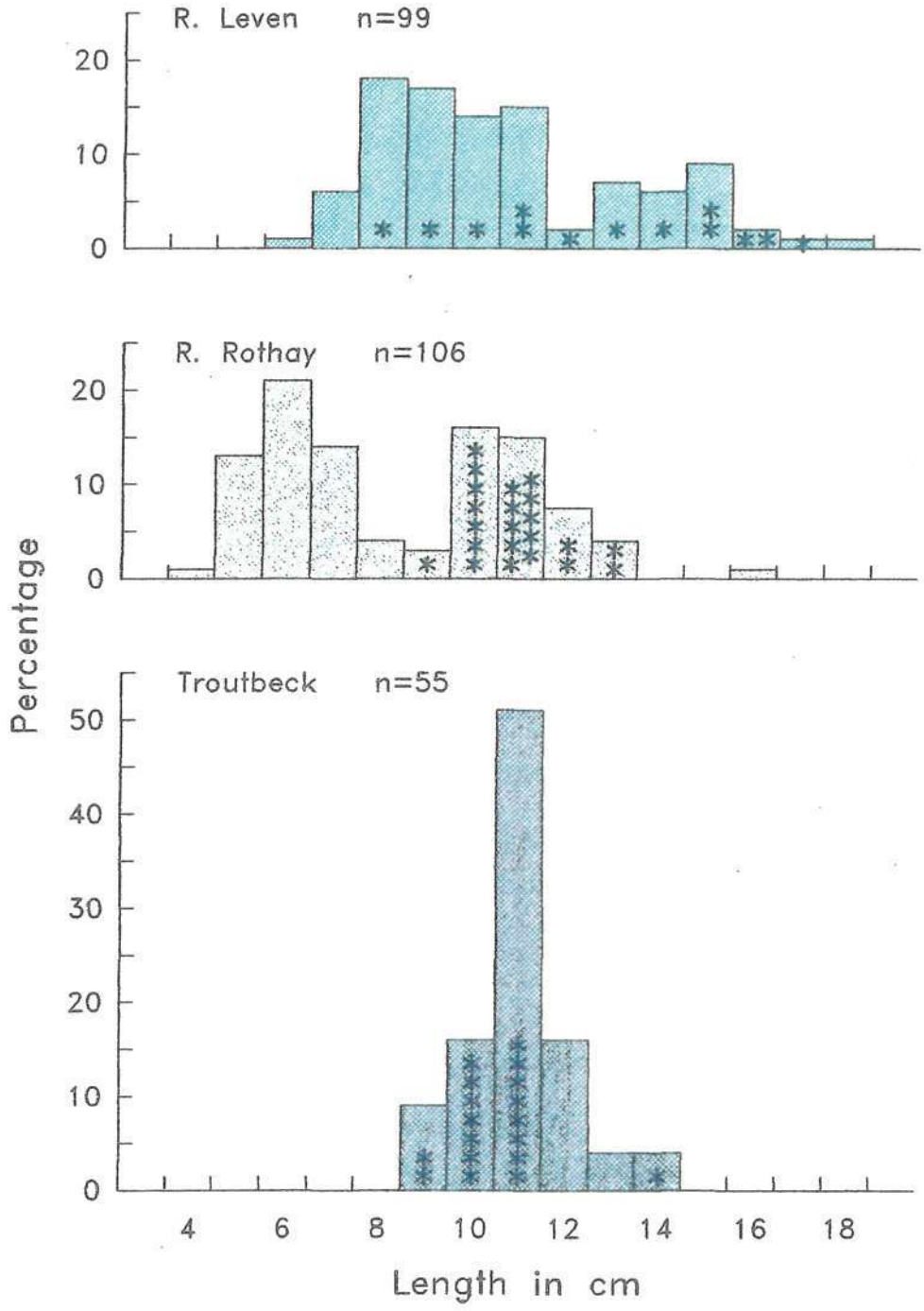

Fig.2 Length distribution of juvenile salmon in the Windermere catchment. Each asterisk denotes a sexually mature male parr.

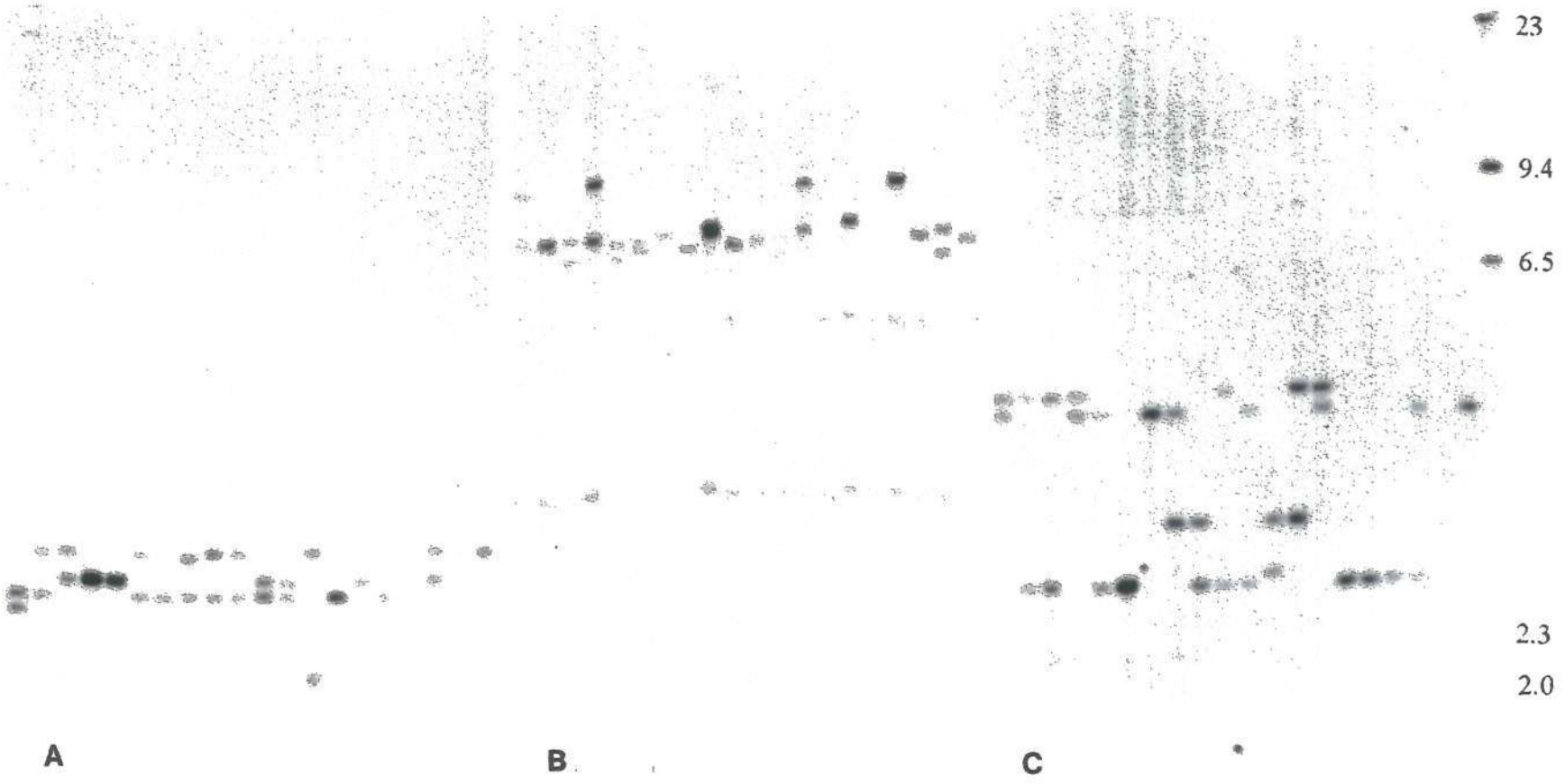

Fig.3 Autoradiograph patterns for twenty Atlantic salmon showing the loci detected by the three single locus probes. A) pSsa-45/1; B) pSsa-45/2; C)pStr-9. The size markers are from *Hin*dIII digested λ DNA.

Table 2: Allele frequencies at locus Ssa-45/1

							New[1]		
Population	*n*	A	C	D	E	F	R	S	T
Troutbeck	45	.400	.000	.400	.022	.000	.000	.100	.078
Leven	86	.448	.326	.157	.047	.006	.006	.012	.000
Rothay	102	.314	.230	.392	.015	.000	.000	.000	.049

1 Alleles not seen in the River Dee system.

Table 3: Allele frequencies at locus Ssa-45/2

													NEW[1]	
Population	*n*	C	D	F	G	H	I	J	K	L	M	N	Q	S	T
Troutbeck	45	.400	.056	.111	.056	.033	.100	.044	.000	.000	.000	.100	.100	.000	.000
Leven	86	.174	.012	.023	.157	.256	.110	.000	.012	.041	.006	.160	.023	.000	.006
Rothay	102	.083	.000	.059	.123	.289	.186	.049	.005	.000	.000	.147	.020	.010	.029
1 Alleles not seen in the River Dee system.															

Table 4: Allele frequencies at locus Str-9

									New[1]
Population	*n*	B	C	D	F	G	H	I	T
Troutbeck	45	.000	.089	.478	.000	.044	.278	.011	.000
Leven	86	.081	.087	.360	.134	.017	.221	.093	.006
Rothay	102	.010	.289	.319	.069	.025	.284	.005	.000
1 Allele not seen in the River Dee system									

Table 5: Genetic variability at all three loci in all three populations with standard errors in parentheses

Population	*n*	pSsa-45/1			pSsa-45/2			pStr-9			Combined		
		No. of Alleles	Heterozygosity		No. of Alleles	Heterozygosity		No. of Alleles	Heterozygosity		Mean No. of Alleles	Heterozygosity	
			Obs.	Exp.		Obs.	Exp.		Obs.	Exp.		Obs.	Exp.
Troutbeck	45	5	.644	.671	9	.778	.797	5	.689	.680	6.3 (1.3)	.704 (.039)	.716 (.041)
Leven	86	7	.744	.670	12	.721	.837	8	.733	.785	9.0 (1.5)	.733 (.007)	.764 (.049)
Rothay	102	5	.696	.695	11	.686	.835	7	.676	.732	7.7 (1.8)	.686 (.006)	.754 (.042)

Table 6: Homogeneity test (contingency table statistics) for observed genotype classes among three Atlantic salmon populations (x^2, df,P).

	Ssa-45/1	
Troutbeck	Leven 70.453, 7, P<<0.0001	Rothay 43.612, 5, P<<0.0001
Leven		42.092, 7, P<<0.0001
	Overall = 99.385, 14, P<<0.0001	
	Ssa-45/2	
Troutbeck	Leven 68.981, 12, P<<0.0001	Rothay 87.565, 11, P<<0.0001
Leven		39.676, 13, P<<0.0002
	Overall = 131.425, 26, P<<0.0001	
	Str-9	
Troutbeck	Leven 24.516, 7, P<<0.0001	Rothay 42.010, 6,P<<0.0001
Leven		53.992, 7, P<<0.0001
	Overall = 85.332, 14, P<<0.001	

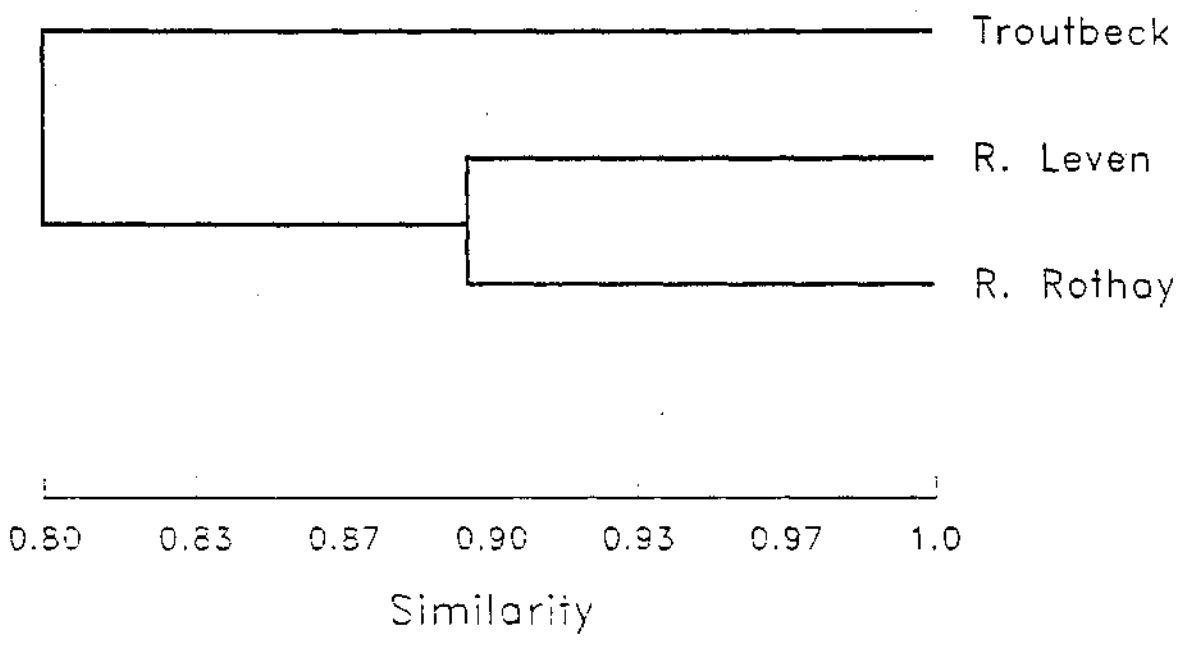

Fig.4 UPGMA dendrogram for the three Windermere salmon populations based on Nei's (1978) unbiased genetic identity.

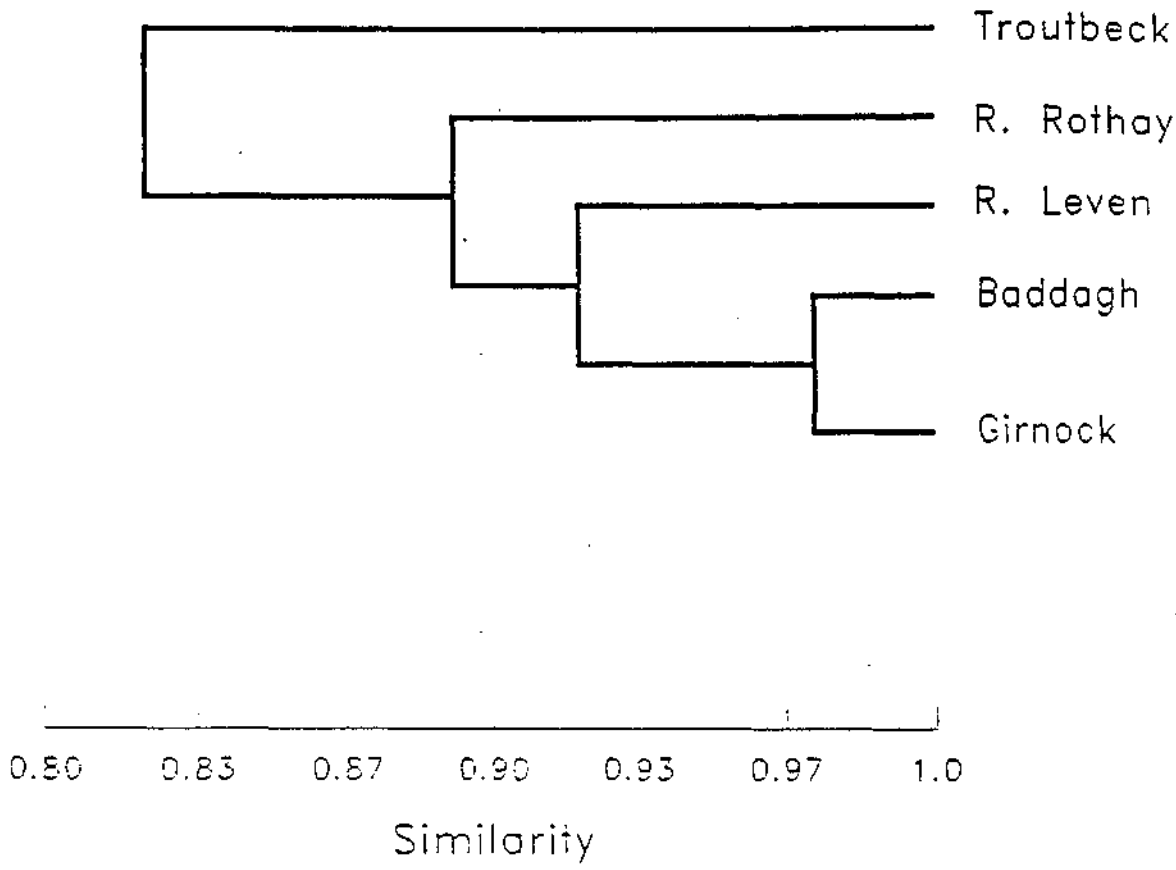

Fig.5 UPGMA dendrogram for three Windermere and two Dee salmon populations based on Nei's (1978) unbiased genetic identity.

Table 8: Atlantic salmon alleles and maternal parent for 10 salmon x trout hybrids from Troutbeck.

Fish	Ssa-45/1	Str-9	F[1]
01	A	D	AS
11	D	I	BT
227	D	I	BT
230	A	G	BT
231	A	H	BT
236	S	H	BT
241	E	C	BT
244	C	G	BT
249	A	I	BT
257	A	I	BT

1 The female parent was determined by examining *Ava*II mt DNA genotypes. Three different brown trout (BT) genotypes were observed.

4. DISCUSSION

4.1 Size distribution and maturity

The length bimodality in the juvenile salmon from the Rivers Leven and Rothay probably represents at least two different year classes (0+ and 1+) within the population. The unimodality of the Troutbeck sample is unexplained but probably represents 1+ fish. Age determination from scale readings or otolith studies would clarify the situation but the procedures are extremely time-consuming (and, therefore, expensive) and the Terms of Reference for the investigation did not allow for such age determination. If the above interpretation of the size bimodality of juvenile salmon in the Rivers Leven and Rothay is correct, one must conclude that the growth rate of salmon in the River Leven is significantly greater than that of salmon in the River Rothay (compare the mean lengths for the lower and upper modes for the two rivers). It is well-established that lakes or impoundments (particularly those that stratify during the summer months) can increase the temperature profile of downstream rivers (Crisp, 1987). Moreover, both the developmental rate and growth rate of salmonid fish are temperature-dependent (see Crisp, 1988; Elliott, 1981 respectively) and it is likely, therefore, that the accelerated growth in the River Leven reflects such a temperature effect. This could arise as a result of earlier emergence from the spawning gravels (effectively increasing the length of the fish's first growing season) or by a direct effect of temperature on growth processes.

Precocious sexual maturity was found in male parr from all sites. The incidence (13-33%) is well within the range found by other workers (see for example Bagliniere and Maisse, 1985) and it is well-accepted that such precocious parr are capable of producing viable sperm and of fertilizing the eggs of adult salmon (the so-called 'sneakers'). However, although the parr can contribute to the population gene pool and then continue to the smolt stage and subsequently migrate to sea (Baglinere

and Maisse, 1985), precocious maturity is associated with an increased mortality rate (Caswell *et al*, 1984). Precocious maturation is influenced by both genetic and environmental factors. Rapid growth during the early life stages favours precocious maturation (see Thorpe, 1989) and this may be achieved during mild winters, at southerly latitudes (elevated temperatures) and with long photoperiod (increased feeding opportunity). Under hatchery conditions, population density may also effect precocious maturation by density-dependent effects on growth rate (Thorpe, 1989) although field data for density effects are more difficult to interpret (Prevost *et al*, 1992). A further school of thought has developed the hypothesis that precocious maturation may be a response to over-exploitation of the adult stock (Caswell *et al*, 1984; Myers and Hutchings, 1987). In the present investigation, it is interesting that precocious males were found in both the presumed 0+ and 1+ age groups in the River Leven (fast growing fish) but only in the presumed 1+ fish from the slower-growing River Rothay fish.

4.2 Observed genetic variation

The study achieved its main objective and clearly demonstrated that the populations of juvenile Atlantic salmon from the Rivers Leven, Rothay and Troutbeck do not form a single, homogeneous population. It is concluded, therefore, that although related, each population should be treated as a separate stock. Indeed the genetic differentiation is larger than might have been expected. Thus from similarity indices based on genomic DNA, salmon from the River Leven would appear to be more similar to fish from the River Dee (Aberdeenshire) than from the River Rothay or Troutbeck! The scientific importance of this finding remains to be fully evaluated and it is argued that efforts must be made to conserve the existing Windermere salmon stocks for scientific purposes. It is likely that artificial relocation of any spawning adults from their natural spawning grounds to other sites within the catchment will jeopardize the genetic integrity of the three stocks and any management

techniques to encourage fish to migrate naturally from the River Leven to the upper catchment are unlikely to succeed. Moreover, any future stocking/enhancement strategy must take into account the results of this study and only enhance each stock from within its own gene pool (if, indeed, any stock enhancement is necessary).

4.3 Hybrids

The frequency of salmon and trout hybrids in the River Leven (1%) is similar to that found in other studies when only one of the two 'species' were sampled (Crozier, 1984; Verspoor, 1988). However, the frequency of hybrids found in the Troutbeck population during the current investigation is extremely high (18%) and is likely to be an underestimate because only fish with a salmon-like morphology were sampled. Hybrid frequencies in excess of 10% have been reported (Jansson *et al*, 1991; McGowan and Davidson, 1992) but only when both the Atlantic salmon and brown trout have been sampled. Evidence from mitochondrial DNA studies shows that the occurrence of hybridization in Troutbeck is not the result of a single, spurious mating but it involves several distinct matings involving both male and female trout and male and female salmon. Although the fertility of hybrids is probably low, the identification of an apparent backcross between an F_1 hybrid and a brown trout in a North American river (Beland *et al*, 1981) suggests that hybrids may, under certain circumstances, contribute to the gene pool. However, the importance of this phenomenon to population control under natural conditions is unknown. Increased hybridization rates are reported to occur when introductions of new species are made (eg brown trout into North America - see Beland *et al*, 1981) or where spawning sites are restricted or degraded (McGowan and Davidson 1992). In the Troutbeck, *Salmo trutta* and *Salmo salar* are both endemic species with a long, sympatric history. Consideration might be given by the NRA to the possibility that the high hybridization rate in Troutbeck is caused by restricted or degraded spawning sites in the river.

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APPENDIX 1 - DETAILS OF INDIVIDUAL FISH

(weight, length, maturity, species, genotype)

APPENDIX 1A - TROUTBECK (FISH 1-19 & 225-260)

Fish No	Weight g	Length cm	State of maturity	Species[2]	Genotype pSsa-45/1	pSsa-45/2	pStr-9
1	18.9	11.9	I	S	DT	DI	HI
2	21.3	13.0	I	H	-	-	-
3	21.5	12.2	I	S	DS	CF	HI
4	19.9	12.0	I	S	AD	CG	DH
5	14.7	10.2	M	S	ST	DG	CD
6	18.6	11.8	I	S	DE	HI	CH
7	18.0	11.8	I	S	ST	CD	DI
8	19.3	11.9	I	S	AA	CG	DD
9	20.8	12.0	I	S	AD	CC	DD
10	14.8	11.1	I	S	AD	CC	CH
11	16.5	11.0	I	H	-	-	-
12	16.5	11.0	M	S	DD	CF	DH
13	12.6	10.1	I	S	AD	HQ	DD
14	21.0	12.0	I	S	AD	IQ	HI
15	19.4	12.0	I	S	AA	CQ	HI
16	13.9	10.2	M	S	AA	GQ	DI
17	10.4	9.9	I	S	DS	FQ	DD
18	22.5	12.5	I	S	AD	IQ	DD
19	20.1	11.6	M	S	AD	CF	DD
225	14.4	11.0	I	S	AD	CC	HH
226	15.2	11.1	I	S	AD	CF	DH
227	30.9	14.1	I	H	-	-	-
228	11.6	10.0	I	S	DD	CF	DD
229	36.0	14.0	M	S	AA	CN	DH
230	8.0	9.2	I	H	-	-	-
231	17.8	11.8	I	H	-	-	-
232	17.1	11.6	I	S	AA	GJ	DI
233	15.6	10.5	M	S	AT	CJ	CG
234	18.8	11.3	M	S	AS	CC	DH
235	17.3	10.9	M	S	DD	NN	HI

APPENDIX 1A continued:

Fish No	Weight g	Length cm	State of maturity	Species[2]	Genotype pSsa-45.1	pSsa-45.2	pStr-9
236	15.6	11.2	I	H	-	-	-
237	17.5	11.5	I	S	AD	CJ	DD
238	17.9	11.7	I	S	ST	CJ	CD
239	17.6	11.4	I	S	DS	FN	HI
240	20.3	11.5	M	S	AD	FN	DD
241	12.3	10.5	I	H	-	-	-
242	20.8	12.1	I	S	DD	CF	DH
243	16.4	11.0	M	S	AA	CN	DD
244	14.1	11.3	I	H	-	-	-
245	14.3	11.0	I	S	DD	CI	CG
246	12.1	10.0	I	S	AT	DN	CD
247	15.6	11.0	M	S	AD	HI	DH
248	15.8	11.2	I	S	AA	CC	DH
249	14.1	11.2	I	H	-	-	-
250	16.9	11.0	I	S	AD	CI	DI
251	15.8	11.2	I	S	DD	CI	GH
252	14.1	10.4	M	S	AD	FN	DD
253	21.2	12.0	M	S	DS	CN	DD
254	18.7	12.0	I	S	DD	CC	HH
255	18.5	11.6	I	S	AA	CC	DH
256	15.1	11.2	I	S	ET	DI	CD
257	8.9	9.5	I	H	-	-	-
258	10.0	9.5	M	S	AA	CC	GH
259	14.4	10.6	M	S	DS	CQ	DH
260	9.5	9.4	M	S	AD	QQ	DH

[1] I = immature
M = mature

[2] S = Salmonid
T = Trout
H = Hybrid

APPENDIX 1B - RIVER LEVEN (FISH 20-118)

Fish No	Weight g	Length cm	State of maturity	Species	Genotype pSsa-45/1	pSsa-45/2	pStr-9
20	14.4	11.0	I	S	AD	GG	DD
21	33.4	13.9	M	S	DD	IN	DD
22	34.3	14.6	I	S	CF	CN	CD
23	44.4	15.8	I	S	CD	KN	HH
24	43.6	15.6	I	S	AA	GG	DI
25	10.7	9.7	I	S	AD	GG	FF
26	15.7	11.1	I	S	CE	CG	DF
27	8.4	9.2	I	S	AC	HL	BD
28	7.4	8.6	I	S	AC	DN	HI
29	8.5	9.3	I	S	CD	NQ	HI
30	4.9	7.8	I	S	AE	CH	DI
31	6.5	8.3	I	S	AC	LQ	BC
32	59.4	16.5	I	S	AC	NN	BD
33	11.1	10.0	I	S	AE	CI	FH
34	12.0	10.4	I	S	AC	GI	CH
35	9.8	9.8	I	S	CD	HN	BD
36	13.3	10.6	I	S	AD	CI	DD
37	9.8	9.9	I	S	AD	IN	DF
38	8.5	9.2	I	S	CC	GH	BH
39	6.4	8.5	I	S	CC	CH	DD
40	3.7	7.0	I	S	AA	HN	FH
41	15.4	11.3	I	S	CC	CC	DD
42	31.7	13.6	I	S	AC	IN	BD
43	11.6	10.2	I	S	CD	FH	BD
44	17.5	11.6	I	S	AD	GI	CT
45	12.7	10.5	I	S	AC	GG	DD
46	37.4	14.6	M	S	AA	GH	CD
47	16.4	11.2	I	S	AC	GG	DF
48	20.4	12.0	M	S	AC	HH	DH

APPENDIX 1B continued

Fish No	Weight g	Length cm	State of maturity	Species[2]	Genotype pSsa-45.1	pSsa-45.2	pStr-9
80	10.4	9.5	I	S	AC	II	DF
81	7.1	8.5	I	S	AS	HN	DD
82	19.6	11.6	M	S	AC	CN	HH
83	48.8	15.5	M	S	AD	NN	DF
84	16.3	11.0	I	S	-	-	-
85	19.3	11.5	I	T	-	-	-
86	7.0	9.3	I	S	CC	CC	HH
87	8.8	9.0	I	S	-	-	-
88	9.3	9.3	I	S	AD	CI	HH
89	6.8	8.3	I	S	AC	CH	HH
90	24.4	13.0	I	S	CC	GG	CH
91	72.1	17.5	M	S	-	-	-
92	36.2	14.5	I	S	CC	CC	CI
93	48.3	16.0	I	S	AD	CH	BC
94	15.0	10.5	I	S	AC	HN	DH
95	12.1	9.5	M	S	CC	HN	BH
96	14.1	10.2	M	S	-	-	-
97	7.8	8.5	I	T	-	-	-
98	9.9	9.0	I	S	AD	HL	FF
99	6.8	8.2	I	S	AA	HH	DH
100	42.0	15.0	I	S	AD	GH	DD
101	8.6	8.7	I	S	AC	GK	CH
102	33.9	14.0	I	S	AA	CH	CD
103	1.3	10.2	I	S	AC	GL	DH
104	9.9	10.0	I	S	AE	HH	CH
105	6.5	8.7	I	S	AD	IT	DH
106	16.9	11.5	I	S	-	-	-
107	15.9	11.0	I	S	CD	HH	CD
108	55.5	15.9	M	S	AC	HH	BD
109	24.9	13.0	I	S	AC	GN	HH

APPENDIX 1B continued

Fish No	Weight g	Length cm	State of maturity	Species[1]	Genotype pSsa-45.1	pSsa-45.2	pStr-9
110	15.1	11.0	I	S	AC	HI	HI
111	8.2	9.0	I	S	-	-	-
112	6.7	8.5	I	S	AA	HH	DH
113	79.3	18.7	I	S	AC	NQ	FF
114	4.1	7.1	I	S	CE	HN	DH
115	12.4	10.4	I	S	AD	CH	FF
116	10.4	9.5	I	S	AD	CI	DD
117	8.3	9.0	I	S	AD	II	DI
118	8.9	9.1	I	S	DR	DN	CH

APPENDIX 1C continued

Fish No	Weight g	Length cm	State of maturity	Species[1]	Genotype pSsa-45.1	pSsa-45.2	pStr-9
149	4.3	7.5	I	S	AT	FH	CD
150	17.6	11.2	M	S	DD	NN	CD
151	4.9	8.0	I	S	AA	II	CG
152	3.3	7.0	I	S	AC	GG	DH
153	4.9	7.9	I	S	CC	HI	CF
154	5.5	8.1	I	S	AA	II	DH
155	3.3	6.9	I	S	DD	HH	CC
156	2.9	6.7	I	S	AA	II	BD
157	3.0	6.6	I	S	AD	HH	CD
158	3.2	6.9	I	S	AC	GI	CC
159	2.4	6.2	I	S	AC	HJ	CH
160	1.4	5.7	I	S	AC	II	CC
161	11.4	10.5	I	S	AC	CI	CH
162	10.5	10.2	I	S	DD	HJ	CH
163	2.1	6.1	I	S	CT	JN	DD
164	2.4	6.0	I	S	AC	CN	DD
165	1.6	5.5	I	S	DT	IN	HH
166	1.4	5.2	I	S	CT	HI	HH
167	26.6	13.0	M	S	AD	HH	CG
168	11.4	10.0	M	S	DD	NQ	DI
169	16.3	11.4	I	S	AD	HH	CD
170	3.0	6.3	I	S	AD	FH	DH
171	24.7	13.0	I	S	CD	GG	DH
172	13.4	10.9	I	S	AD	HI	HH
173	2.8	6.6	I	S	DD	CI	CD
174	17.7	12.0	I	S	AD	CN	DH
175	2.2	6.0	I	S	AD	CH	DF
176	1.7	5.6	I	S	DE	CI	DH
177	2.4	6.3	I	S	AD	GH	DD

APPENDIX 1C continued

Fish No	Weight g	Length cm	State of maturity	Species	Genotype pSsa-45/1	pSsa-45/2	pStr-9
178	16.0	11.0	M	S	DE	HN	DD
179	26.3	13.2	M	S	AC	NT	DF
180	18.1	11.6	M	S	CT	FH	CG
181	15.4	11.0	M	S	AD	NN	DD
182	4.6	7.6	I	S	AA	CH	DF
183	2.5	6.2	I	S	AD	CT	DF
184	16.4	11.1	M	S	AD	GG	DH
185	1.4	5.3	I	S	AC	JT	FF
186	16.2	11.5	I	S	CD	GJ	CC
187	14.8	11.2	I	S	AC	CI	CC
188	13.6	11.0	I	S	AA	FN	DH
189	12.0	10.4	I	S	CD	NN	DD
190	10.3	9.9	M	S	AD	GN	CH
191	1.7	5.5	I	S	DT	CC	CD
192	1.7	5.6	I	S	AC	TT	FH
193	0.8	4.8	I	S	AD	HH	HH
194	1.7	5.7	I	S	DT	FT	CH
195	22.2	12.5	I	S	-	-	-
196	20.7	12.6	I	S	-	-	-
197	11.2	10.0	I	S	DD	GG	CC
198	2.4	6.2	I	S	CD	GJ	CH
199	17.3	11.5	M	S	AC	FH	HH
200	13.3	10.5	M	S	AC	GH	CD
201	15.2	11.5	I	S	DD	CC	CH
202	2.4	6.0	I	S	CD	FH	CH
203	3.4	7.0	I	S	-	-	-
204	20.1	12.0	M	S	DT	HI	HH
205	4.9	7.9	I	S	AD	FI	DH
206	3.4	6.8	I	S	AC	HH	CH
207	9.4	9.6	I	S	CC	HI	CD

APPENDIX 1C continued

Fish No	Weight g	Length cm	State of maturity	Species*	Genotype pSsa-45.1	pSsa-45.2	pStr-9
208	8.8	8.4	I	S	CC	HN	CC
209	5.2	7.9	I	S	AD	HI	CD
210	2.5	6.0	I	S	DD	HH	CH
211	4.0	7.1	I	S	AD	II	CH
212	14.9	10.3	M	S	AD	HH	CD
213	16.5	11.5	I	S	AD	HN	DH
214	18.9	12.3	I	S	CD	HJ	HH
215	11.0	10.0	I	S	CD	GI	DD
216	1.8	5.5	I	S	AE	IS	DF
217	5.5	8.1	I	S	DD	II	FH
218	3.9	7.0	I	S	CC	HH	CH
219	3.2	6.6	I	S	DD	GH	DH
220	4.7	7.5	I	S	AD	IN	FG
221	4.9	7.5	I	S	AC	HN	CF
222	2.3	5.9	I	S	AT	II	CD
223	4.0	7.2	I	S	AA	HI	HH
224	1.9	5.6	I	S	CD	HH	HH