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Selective exploitation of early running fish may induce genetic and phenotypic changes in Atlantic salmon

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Genetic evidence for the selective exploitation by anglers of early running fish was examined in four Iberian Atlantic salmon populations using protein and mtDNA markers. The populations studied had been exploited exclusively by anglers since 1949 during a fixed fishing season that ran approximately from March to July. Genetic variation at six protein loci was small and was accounted for by the *MEP-2** and *MDH-3,4** polymorphisms, which generally remained stable over time and were in Castle–Hardy–Weinberg equilibrium during the fishing season. Early running fish that had spent multiple winters at sea (MSW) generally had higher frequencies of the common *MEP-2** (100) allele than did late running, one sea winter (1SW) grilse that were significantly smaller and tended to escape the fishery. Spawners differed from angler caught fish in their mtDNA frequencies and consistently had a lower sea-age and a smaller body size. Spawners also smolted at an older age and displayed lower frequencies of the *MEP-2** (100) allele in three of the four populations studied. These results suggest that in these rivers anglers selectively exploit a distinct component of the population and inadvertently cause a differential mortality of genetic types that is likely to be detrimental to population viability.

Key words: Atlantic salmon; conservation; isozymes; mtDNA; population structuring; selective exploitation.

INTRODUCTION

Many commercially valuable fish species, including salmonids, are exploited seasonally to give protection from fishing at vulnerable life history stages or during spawning, or a better chance to reproduce. The side effects of seasonal fishing closures, however, have seldom been considered. For example, despite clear indications that salmonids are structured both spatially and temporally (Hansen & Jonsson, 1991; Adams *et al.*, 1994; Ferguson *et al.*, 1995), some

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populations of Atlantic salmon *Salmo salar* L. continue to be exploited in many rivers during only a few months of the year, typically from early spring until summer. Consequently, some individuals or components of the population may be subjected to higher mortality rates than others, and a certain degree of artificial selection may occur.

In the case of Atlantic salmon, adults that enter the rivers in the spring are often dominated by large multi sea-winter (MSW) females, whereas fish that enter rivers in late summer and fall are often dominated by male-biased, one sea-winter grilse (Gardner, 1976; Shearer, 1992). Since traits such as age at maturity or timing of entry into the rivers are partially inherited and likely to be adaptive (Hansen & Jonsson, 1991; Taylor, 1991; Stewart *et al.*, 2002), there is considerable scope for differential mortality of genetic types and, hence, a definite risk of inadvertent artificial selection by anglers.

Exploited salmonid populations have been shown to respond to differential mortality in several ways. For example, when the fishery is concentrated upon the largest individuals (Gee & Milner, 1980), fish populations may respond by decreasing their average body size or by maturing at an earlier sea-age (Thorpe, 1993). Other phenotypic responses attributed to exploitation in Atlantic salmon include a shift towards delayed entry into rivers (García de Leániz *et al.*, 1992) or a greater incidence of sexually mature male parr (Caswell *et al.*, 1984). In contrast, it has proved much more difficult to detect explicit genetic effects that are attributable to fisheries perhaps due to the low resolution of the molecular markers employed, which may only cover a small part of the genome (Conover & Munch, 2002). Consequently, the role of fishing in changing the genetic structure of fish populations remains controversial (Smith *et al.*, 1991; Policansky, 1993; Hutchings, 2001; Law, 2001).

In the present study, protein and mitochondrial DNA variation were examined in relation to population structuring and differential exploitation in endangered Atlantic salmon populations from Cantabria (N. Spain). Evidence for selective exploitation was assessed by comparing genetic and phenotypic traits between (1) early and late running fish and (2) fish caught by anglers and fish that survived to spawn.

MATERIALS AND METHODS

STUDY RIVERS AND COLLECTION OF SAMPLES

The four small populations studied (rivers Asón, Pas, Nansa, and Deva) have been exploited exclusively by rod and line anglers since 1949 over a fairly constant fishing season, typically extending from mid-March to mid-July (García de Léaniz & Martinez, 1988; García de Leániz *et al.*, 1989). Average yearly rod and line catches during 1988–2000 ranged from $48 \pm 61{\cdot}5$ for the River Deva to $108 \pm 135{\cdot}9$ for the River Pas. Samples of muscle tissue and adipose fins were obtained from nearly all fish caught by anglers during the period 1988–2000, as well as from a sample of spawners and adults that escaped the fishery during the same period. Liver tissue was collected from a smaller sub-sample of fish. Most spawners were collected in adult fish traps located in the same, short (<40 km) accessible reaches of the main rivers where angling took place, or by electrofishing. Data for the River Nansa indicated that there was no size difference between spawners caught by electrofishing and spawners caught in fish traps (Mann–Whitney, $P = 0{\cdot}944$). Fork length, L_F (cm) and wet mass (g) were recorded from most

fish and samples of scales taken for age determination. Fish were classified as grilse (one winter at sea) or multiple sea winter (MSW; 2–3 winters at sea) based on scale reading or (when scales were not available or could not be read) according to their L_F, using 70 cm as the cut-off point between both age groups. Hatchery-reared fish (all identified by fin-clipping since 1992) were excluded from analyses, as these are known to differ from wild individuals (Verspoor, 1988, 1994).

PROTEIN ELECTROPHORESIS

The following isozymes were screened in skeletal muscle: NADP+ dependent malic enzyme (MEP-2; EC.1.1.1.40), malate dehydrogenase (MDH-3,4, EC.1.1.1.37), phospho-glucomutase (PGM-1, EC.5.4.2.2) and glucose-6-phosphate isomerase (GPI-1,2, EC.5.3.1.9). Lactate dehydrogenase (LDH-4, EC.1.1.1.27) and isocitrate dehydrogenase (IDHP-3, EC.1.1.1.42) were screened in liver. These loci were examined because previously they had been shown to be polymorphic in Iberian salmon populations (García de Leániz *et al*., 1989; Sánchez *et al*., 1991, 1996; Morán *et al*., 1994). Horizontal starch gel electrophoresis was carried out according to Verspoor (1988) using the buffer system citrate-aminomorpholine described in Clayton & Tretiak (1972), adjusted to pH 6·6 (Verspoor & Cole, 1989) and the Tris-citrate-borate system as described in Verspoor (1988). Protein designation follows Shaklee *et al*. (1989). The most common allele is designated 100 and other alleles were scored according to their relative electrophoretic mobility. Loci that proved to be monomorphic in a preliminary screening were excluded from the analysis. Departure from Castle-Hardy-Weinberg equilibrium was tested using the GENEPOP package (v. 3.2, Raymond & Rousset, 1995). Homogeneity of allele frequencies among year classes was tested using the Fisher's exact test. Differences in genotype frequencies between grilse and MSW fish, as well as between spawners and angler caught fish, were tested for statistical significance with an unbiased log-likelihood test.

mtDNA ANALYSIS

Total DNA was extracted from the adipose fins (Taggart *et al*., 1992) of 673 wild adult salmon collected during 1996–2000. One µl of the extracted DNA was used to amplify a 1400 bp fragment in the ND1 region with the 16sRNA (Palumbi, 1996) and ND1 (Cronin *et al*., 1993) primers, followed by digestion with the restriction enzymes *HaeIII, HinfI, RsaI* and *DraI* (Verspoor *et al*., 1999). Differences in haplotype composition between spawners and fish caught by anglers were examined using the MONTE program in the REAP software package (McElroy *et al*., 1991).

RESULTS

PROTEIN VARIATION

Of the six protein loci that were screened, only two loci (*MEP-2** and *MDH-3,4**) were found to be variable in Cantabrian populations (Table I). All rivers had high frequencies of the common **100* allele for both loci, although no significant association was detected between *MEP-2** and *MDH-3,4** genotypes ($G = 2{\cdot}754$, d.f. $= 2$, $n = 863$, $P = 0{\cdot}252$). Heterogeneity analysis between the four populations, on the other hand, indicated the existence of spatial structure at the two polymorphic loci (*G*-test on allele counts; *MEP-2**, $\chi^2 = 21{\cdot}80$, d.f. $= 3$, $P < 0{\cdot}001$; *MDH-3,4**, $\chi^2 = 56{\cdot}48$, d.f. $= 3$, $P < 0{\cdot}001$).

TABLE I. Protein variation among wild adult Atlantic salmon returning to Cantabrian rivers during the period 1988–2000, excluding known hatchery reared fish (identified by fin-clipping). Sample size (in parentheses) and frequency of the common allele are shown for each locus

	Locus					
River	*MEP-2**	*GPI-1,2**	*LDH-4**	*IDHP-3**	*MDH-3,4**	*PGM-1**
Asón	0·917*	1·000	1·000	1·000	0·989	1·000
	(682)	(24)	(24)	(24)	(417)	(71)
Pas	0·966	1·000	1·000	1·000	0·909	1·000
	(294)	(4)	(4)	(4)	(121)	(122)
Nansa	0·939*	1·000	1·000	1·000	0·949	1·000
	(469)	(13)	(13)	(13)	(236)	(106)
Deva	0·954*	1·000	1·000	1·000	0·891	1·000
	(346)	(7)	(7)	(7)	(110)	(86)

*Significant deviations from CHW.

DEPARTURES FROM CASTLE–HARDY–WEINBERG EQUILIBRIUM

All year classes that contributed to the rod and line fisheries were found to be in Castle–Hardy–Weinberg equilibrium for both loci in the four rivers studied (Tables II and III). Heterozygote deficiencies were detected for *MEP-2** in the River Asón in the 1993 year class ($P = 0{\cdot}026$) and for *MDH-3,4** in the River Nansa in the 1996 year class ($P = 0{\cdot}010$), but these were not significant after adjusting for multiple testing using a Bonferroni correction.

TEMPORAL STABILITY IN GENOTYPE FREQUENCIES

Genotype frequencies during the fishing season were stable over time for both *MEP-2** (Table II) and *MDH-3,4** (Table III). No significant heterogeneity in genotype frequencies was found between year classes in the rivers Asón, Nansa and Deva. Only the River Pas had significant temporal heterogeneity for *MEP-2** genotype frequencies, due in part to lack of variation in two consecutive year classes (1995 and 1996, Table II).

INFLUENCE OF *MEP-2** ON SEA-AGE AND FISH SIZE

Analysis of sea-age (Table IV) indicated that the *MEP-2** genotype was associated with the time adults spent at sea in the River Asón ($P = 0{\cdot}003$), River Nansa ($P = 0{\cdot}040$) and perhaps also in the River Deva ($P = 0{\cdot}052$). In every case, analysis of the standardized residuals indicated that fish that were homozygous for the **100* allele tended to mature later (*i.e.* they were MSW salmon) than did fish with the **125* allele that were more likely to become grilse. Combined probabilities across rivers using Fisher's exact test indicated that the observed association between *MEP-2** genotype and sea-age is unlikely to be an artifact ($\chi^2 = 24{\cdot}62$, d.f. $= 8$, $P = 0{\cdot}0018$).

TABLE II. Distribution of *MEP-2** genotypes among wild adults sampled in the rod and line fisheries (1988–2000) by year classes. Temporal stability in genotype frequencies and departures from Castle–Hardy–Weinberg (CHW) equilibrium are indicated

	*MEP-2** genotype						
Year class	*100/100*	*100/125*	*125/125*	Total	F_{IS}	CHW	Frequency (100)
River Asón, $P = 0{\cdot}227$							
1983	1	0	0	1	–	–	–
1984	18	4	0	22	−0·080	NS	0·925
1985	183	19	4	206	+0·249	NS	0·934
1986	22	1	0	23	−0·012	NS	0·979
1987	16	4	0	20	−0·111	NS	0·900
1988	15	4	1	20	+0·216	NS	0·850
1989	1	0	0	1	–	–	–
1991	4	1	0	5	0·000	NS	0·900
1992	27	6	1	34	+0·165	NS	0·882
1993	73	18	5	96	+0·252	NS	0·854
1994	27	4	0	31	−0·053	NS	0·935
1995	21	1	1	23	+0·656	NS	0·935
1996	19	3	0	22	−0·050	NS	0·932
1997	3	0	0	3	–	–	–
River Nansa, $P = 0{\cdot}835$							
1984	10	3	0	13	−0·130	NS	0·885
1985	34	1	0	35	−0·010	NS	0·986
1986	19	2	1	22	+0·450	NS	0·909
1987	2	0	0	2	0·000	–	–
1992	5	1	0	6	−0·091	NS	0·917
1993	57	4	1	62	+0·299	NS	0·952
1994	29	3	0	32	−0·049	NS	0·953
1995	21	3	0	24	−0·067	NS	0·938
1996	18	2	0	20	−0·053	NS	0·950
1997	27	3	1	31	+0·347	NS	0·919
1998	10	1	0	12	−0·091	NS	0·917
River Pas, $P = 0{\cdot}010$							
1991	1	0	0	1	–	–	–
1992	19	1	0	20	−0·026	NS	0·975
1993	70	7	0	77	−0·048	NS	0·955
1994	11	3	1	15	+0·280	NS	0·833
1995	16	0	0	16	0·000	NS	1·000
1996	16	0	0	16	0·000	NS	1·000
1997	49	1	0	50	−0·010	NS	0·990
River Deva, $P = 0{\cdot}205$							
1992	6	2	0	8	−0·143	NS	0·875
1993	181	14	2	197	+0·187	NS	0·954
1994	26	0	0	26	0·000	NS	1·000
1995	4	0	0	4	0·000	NS	1·000
1996	7	1	0	8	−0·067	NS	0·937
1997	65	4	0	69	−0·030	NS	0·971

TABLE III. Distribution of *MDH-3,4** genotypes among wild adults sampled in the rod and line fisheries by year classes. Temporal stability in genotype frequencies and departures from Castle–Hardy–Weinberg (CHW) equilibrium are indicated

	*MDH-3,4** genotype						
Year class	*100/100*	*100/80*	*80/80*	Total	F_{IS}	CHW	Frequency (100)
River Asón, $P = 0{\cdot}164$							
1983	1	0	0	1	–	–	–
1984	22	0	0	22	0·000	NS	1·000
1985	205	2	0	207	−0·002	NS	0·995
1986	23	0	0	23	0·000	NS	1·000
1987	14	0	0	14	0·000	NS	1·000
1988	10	0	0	10	0·000	NS	1·000
1993	9	0	0	9	0·000	NS	1·000
1994	25	0	0	25	0·000	NS	1·000
1995	20	2	0	22	−0·024	NS	0·955
1996	21	1	0	22	−0·000	NS	0·977
1997	3	0	0	3	–	–	–
River Nansa, $P = 0{\cdot}053$							
1984	13	0	0	13	0·000	NS	1·000
1985	35	0	0	35	0·000	NS	1·000
1986	22	0	0	22	0·000	NS	1·000
1987	2	0	0	2	–	–	–
1994	2	0	0	2	–	–	–
1995	7	1	0	8	−0·067	NS	0·938
1996	17	1	2	20	+0·771	NS	0·875
1997	25	6	0	31	−0·107	NS	0·903
1998	9	3	0	12	−0·143	NS	0·875
River Pas, $P = 0{\cdot}180$							
1996	4	4	0	8	−0·333	NS	0·750
1997	45	10	0	55	−0·102	NS	0·909
River Deva, $P = 0{\cdot}602$							
1992	0	1	0	1	–	–	–
1993	10	3	0	13	−0·130	NS	0·885
1994	4	1	0	5	−0·111	NS	0·900
1995	4	0	0	4	0·000	NS	1·000
1996	5	2	0	7	−0·167	NS	0·857
1997	49	16	0	65	−0·133	NS	0·877

To dissociate the effect of the *MEP-2** genotype on the sea-age and size of returning adults, and due also to the small number of *MEP-2* 125/125* homozygotes present in Cantabrian populations, data from the four rivers were pooled together. Fish that were homozygous for the *MEP-2* 100* allele were significantly larger (Kruskal–Wallis, $P = 0{\cdot}001$), heavier (Kruskal–Wallis, $P = 0{\cdot}002$) and remained longer at sea (Kruskal–Wallis, $P < 0{\cdot}001$) than fish that were homozygous for the *MEP-2* 125* allele, whereas heterozygotes were intermediate in size and sea-age (Figs 1 and 2). Within each age class, however, no difference existed between *MEP-2** genotype and L_F for either grilse

TABLE IV. Distribution of *MEP-2** and *MDH-3,4** genotypes among wild grilse and MSW adults sampled during the 1988–2000 runs. Probabilities refer to a Fisher's exact test for differences in allelic frequencies

	*MEP-2** genotype				
River/Age Class	*100/100*	*100/125*	*125/125*	Total	Frequency (100)
River Asón, $P = 0{\cdot}003$					
Grilse	75	17	7	99	0·843
MSW	477	64	8	549	0·927
River Pas, $P = 0{\cdot}722$					
Grilse	30	1	0	31	0·984
MSW	242	17	1	260	0·963
River Nansa, $P = 0{\cdot}040$					
Grilse	147	21	4	172	0·916
MSW	264	18	3	285	0·958
River Deva, $P = 0{\cdot}052$					
Grilse	28	1	2	31	0·919
MSW	291	21	3	315	0·957
	*MDH-3,4** genotype				
River/Age Class	*100/100*	*100/80*	*80/80*	Total	Frequency (100)
River Asón, $P = 0{\cdot}607$					
Grilse	58	2	0	60	0·967
MSW	349	7	0	356	0·980
River Pas, $P = 0{\cdot}181$					
Grilse	13	3	1	17	0·853
MSW	85	17	0	102	0·917
River Nansa, $P = 0{\cdot}423$					
Grilse	70	9	0	79	0·943
MSW	134	11	2	147	0·949
River Deva, $P = 0{\cdot}121$					
Grilse	11	0	0	11	1·000
MSW	75	24	0	99	0·879

(Kruskal–Wallis, $P = 0{\cdot}589$) or MSW (Kruskal–Wallis, $P = 0{\cdot}609$). This suggested that the *MEP-2** genotype was primarily associated with age at maturity and that this, in turn, determined the size attained by returning fish. Thus, average grilsing rates in the four populations increased from 17·3% for *MEP-2* 100/100* to 47·9% for *MEP-2* 125/125*, while the incidence of grilse among *MEP-2* 100/125* was intermediate (21·2%).

No significant association was found between the *MEP-2** genotype and freshwater age (Kruskal–Wallis, $P = 0{\cdot}208$) or between the *MDH-3,4** genotype (pooling together fish with the **80* allele) and L_F (Kruskal–Wallis, $P = 0{\cdot}367$), sea-age (Kruskal–Wallis, $P = 0{\cdot}856$), or freshwater age (Kruskal–Wallis, $P = 0{\cdot}683$) of returning adults.

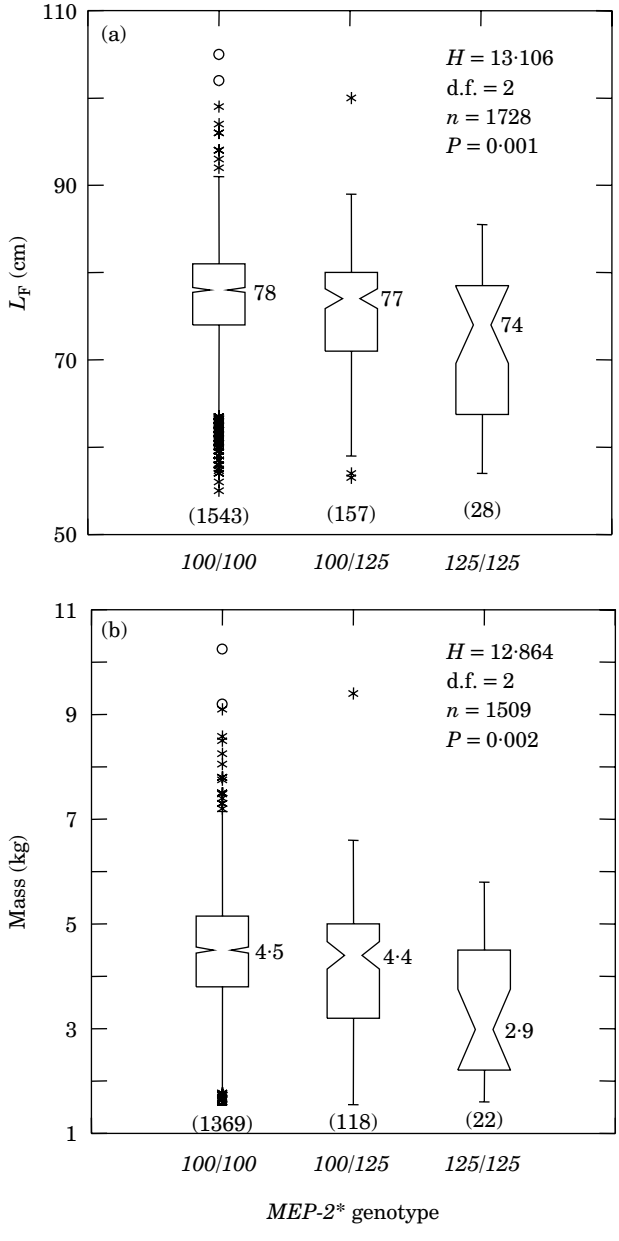

FIG. 1. Relationship between *MEP-2** genotype and variation in (a) L_F (cm) and (b) wet mass (kg) of adult salmon returning to Cantabrian streams (including both fish caught by anglers and spawners), tested for statistical significance by the Kruskal–Wallis H-statistic. Box and whisker plots show median values, first and third quartiles (50% of values) and extreme values (○). Notches extend to 95% CIs around the median, shown in numbers. Sample size in parentheses.

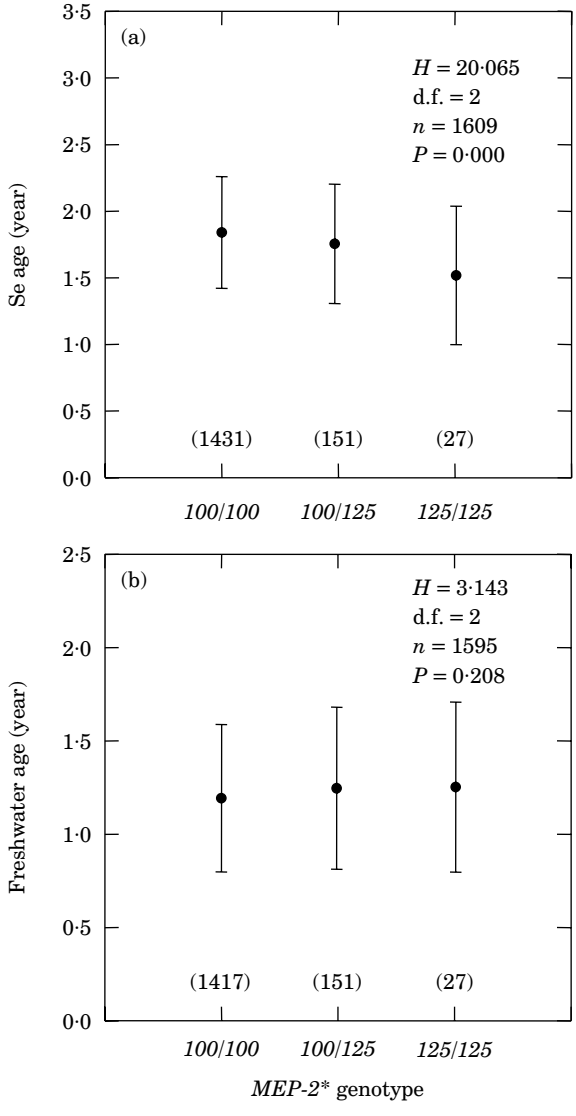

FIG. 2. Relationship between *MEP-2** genotype and variation in (a) sea age (yr) and (b) freshwater age (yr) of adult salmon (fishery + spawners) returning to Cantabrian streams (means, S.D.), tested for statistical significance by the Kruskal–Wallis *H*-statistic. Sample size in parentheses.

PHENOTYPIC AND GENETIC DIFFERENCES BETWEEN SPAWNERS AND FISH CAUGHT BY ANGLERS

Data for Cantabrian populations indicated that the average size and sea-age of returning adults decreased markedly as the season progressed (Fig. 3). Consequently, salmon caught by anglers during the fishing season (mid-March to mid-July) were significantly larger and matured at an older sea-age than salmon that survived to spawn in all four populations (Table V). Compared to

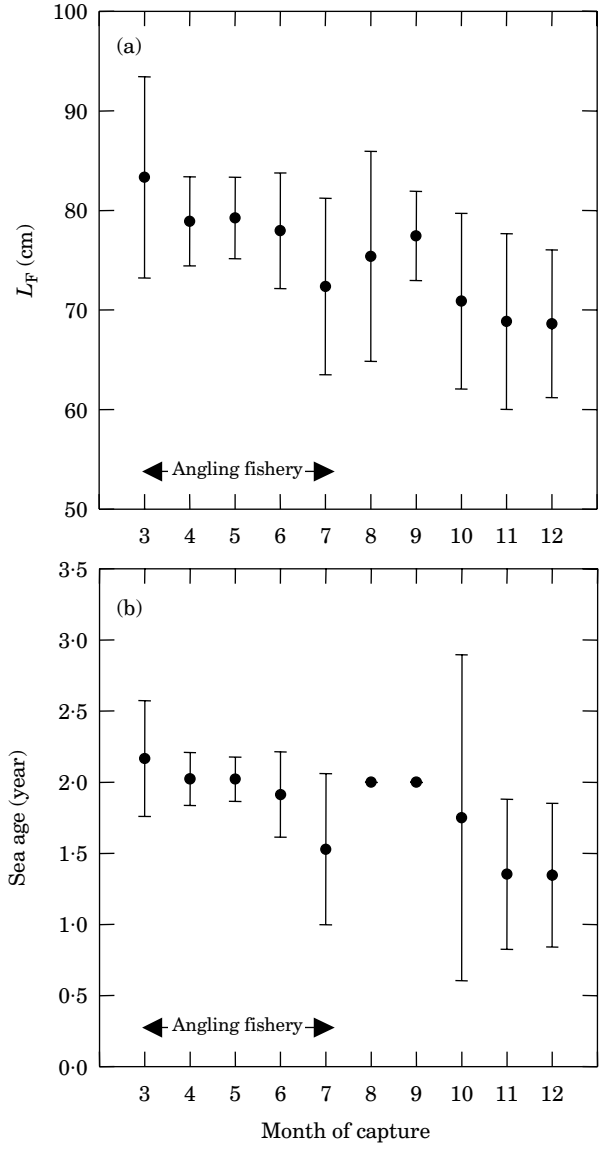

FIG. 3. Monthly variation (mean, S.D.) in L_F (cm, $n = 1720$) and sea-age (yr, n = 1600) of wild adult salmon (fishery + spawners) returning to Cantabrian rivers during the period 1988–2000. The extent of the angling season is indicated.

angler caught fish, spawners also tended to smolt at a significantly older age in three of the four populations.

Genetic differences between angler caught fish and spawners were also evident at isozyme and mtDNA levels, which again indicated the existence of selective exploitation by the fishery. Thus spawners had a higher frequency of the *MEP-2* 125* allele than did fish caught by anglers in three of the four populations studied. Although such differences were only statistically significant in the River

TABLE V. Demographic and genetic differences between spawners and fish caught by anglers in four Iberian Atlantic salmon populations (means ± S.E.; sample size in parentheses). Probabilities refer to the null hypotheses of no difference in age or body size (tested by the non-parametric Kruskal–Wallis) or in allele/haplotypic frequencies (tested by a Fisher's exact test)

				*MEP-2**				*MDH-3,4**				mtDNA haplotype *HaeIII, HinfI, RsaI, DraI*					
	Freshwater age (year)	Sea-age (year)	L_F (cm)	*100/100*	*100/125*	*125/125*	Frequency (100)	*100/100*	*100/80*	*80/80*	Frequency (100)	AAAB	AABB	DABB	DBBB	DAAB	Frequency (AAAB)
River Asón																	
Fishery	1·25 ± 0·02 (517)	1·93 ± 0·01 (523)	77·0 ± 0·23 (552)	489	67	12	0·920 (568)	356	6	0	0·992 (362)	59	26	1	0	0	0·686 (86)
Spawners	1·36 ± 0·06 (76)	1·40 ± 0·06 (76)	70·3 ± 0·80 (101)	95	16	3	0·903 (114)	52	3	0	0·973 (55)	58	8	0	0	0	0·879 (66)
P	0·045	0·000	0·000				0·749				0·118						0·007
River Pas																	
Fishery	1·19 ± 0·03 (204)	2·00 ± 0·01 (207)	78·7 ± 0·28 (229)	206	14	1	0·964 (221)	62	17	0	0·892 (79)	95	0	3	0	1	0·960 (99)
Spawners	1·37 ± 0·07 (60)	1·62 ± 0·07 (61)	72·2 ± 1·02 (70)	69	4	0	0·973 (73)	38	3	1	0·940 (42)	73	6	4	0	0	0·880 (83)
P	0·007	0·000	0·000				1·000				0·251						0·015
River Nansa																	
Fishery	1·11 ± 0·02 (259)	1·86 ± 0·03 (261)	78·1 ± 0·47 (285)	258	24	3	0·947 (285)	133	12	2	0·946 (147)	105	6	1	0	0	0·929 (112)
Spawners	1·23 ± 0·03 (159)	1·30 ± 0·04 (159)	67·9 ± 0·57 (168)	161	19	4	0·927 (184)	81	8	0	0·955 (89)	101	22	4	0	0	0·795 (127)
P	0·001	0·000	0·000				0·013				0·688						0·003
River Deva																	
Fishery	1·16 ± 0·02 (313)	1·97 ± 0·01 (315)	79·9 ± 0·27 (318)	297	21	2	0·961 (320)	73	23	0	0·880 (96)	78	0	1	0	0	0·987 (79)
Spawners	1·15 ± 0·07 (26)	1·31 ± 0·09 (26)	67·6 ± 1·34 (26)	22	1	3	0·865 (26)	13	1	0	0·964 (14)	16	3	1	1	0	0·762 (21)
P	0·971	0·000	0·000				0·009				0·329						0·002

Deva ($P = 0{\cdot}009$) and River Nansa ($P = 0{\cdot}013$; Table V), they were significant if considered collectively across the four rivers (Fisher's exact test for combined probabilities, $\chi^2 = 18{\cdot}68$, d.f. $= 8$, $P = 0{\cdot}016$). No such difference between spawners and angler caught fish was evident with respect to *MDH-3,4** allele frequencies (Fisher's test of combined probabilities, $\chi^2 = 10{\cdot}01$, d.f. $= 8$, $P = 0{\cdot}251$).

Data from mitochondrial DNA indicated that there were significant genetic differences between angler caught fish and spawners in all four populations (Table V). In three of the populations, fish caught by anglers had a higher frequency of the most common AAAB haplotype, while in the River Asón the opposite trend (lower frequency of the common haplotype amongst spawners) was observed.

DISCUSSION

Cantabrian populations, in common with other Atlantic salmon populations (Davidson *et al.*, 1989), appear to have limited genetic variation at the protein level. Of the six loci screened, only two loci (*MEP-2** and *MDH-3,4**) were found to be polymorphic and the level of variation was small, typically with frequencies of the rarer allele $<0{\cdot}10$. Moreover, unlike other, larger Iberian salmon populations (Sánchez *et al.*, 1991, 1996; Morán *et al.*, 1994), salmon in Cantabrian rivers seem to be monomorphic for *IDHP-3**, *PGM-1**, *LDH-4**, and *GPI-1,2*, though our sample sizes were small. Mitochondrial DNA variation, on the other hand, appears to be relatively high, and this probably reflects the ancient, pre-glacial origin of Atlantic salmon in the Iberian glacial refugium (Consuegra *et al.*, 2002).

The populations studied appear to be stable over time at the two polymorphic protein loci examined, in some cases for up to 14 years. Temporal stability at protein loci is common in many Atlantic salmon populations (Crozier & Moffett, 1989; Jordan *et al.*, 1992; Moffett & Crozier, 1996) and this has been associated with high levels of gene flow and large effective population sizes (Jordan *et al.*, 1992). Temporal stability at protein loci could also indicate the existence of selection (Endler, 1986), however, as appears to be the case for the malic enzyme (*MEP-2**) in Atlantic salmon (Verspoor & Jordan, 1989; Jordan & Youngson, 1991; Verspoor *et al.*, 1991). If so, the temporal stability observed in Iberian populations may be more indicative of intense directional selection in extreme, marginal habitats, than of large population sizes and elevated levels of gene flow.

At a finer temporal scale, our results indicate that there is an important degree of seasonal structuring with respect to both phenotypic (body size, sea-age) and genetic (*MEP-2**, mtDNA) traits. Body size and sea-age were found to decrease markedly as the season progressed, and both traits appeared to parallel the frequency of *MEP-2** genotype. Thus, fish that were homozygous for the *MEP-2* (100)* allele remained longer at sea and tended to enter the rivers earlier than did fish with the **125* allele, which in Cantabrian rivers were more likely to mature as grilse and entered the rivers later in the season. Fish that were homozygous for the **100* allele of *MEP-2** were also significantly larger and heavier than **125* homozygotes, while heterozygotes were intermediate in size. Such differences in body size, however, are largely due to the association of

TABLE VI. Predicted phenotypic and genetic changes that might occur in Cantabrian Atlantic salmon populations that result from the selective exploitation by anglers of early running fish (based on the observed differences between angler caught fish and spawners described in Table V) and historical changes observed in the same populations during the period 1945–2000

Trait	Predicted change	Observed change (1945–2000)	Reference
1. Run timing	Shift towards delayed entry	Median timing of capture in the angling fishery delayed by 12–29 days	García de Leániz *et al.* (1992, 2001)
2. Adult size	Reduction in body size	Average size of angler caught fish decreased by 2–6% in length and 10–15% in mass	García de Leániz *et al.* (1992, 2001)
3. Age structure	Increased smolt age, lower sea-age, increased incidence of grilse	Widespread increase in the incidence of grilse in catch	García de Leániz *et al.* (1992, 2001)
4. Life span	Reduced longevity	Reduced life span and almost complete loss of repeated spawners in all rivers	García de Leániz *et al.* (1992, 2001)
5. *MEP-2**	Reduced frequency of the common (**100*) allele	Frequencies generally stable over time (1988–2000)	This publication (Table II)
6. mtDNA ND1/16sRNA	Change in haplotypic frequencies	Significant temporal change in mtDNA haplotypic frequencies in the R. Asón	Consuegra *et al.* (2001, 2002)

*MEP-2** with age at maturity, which seems to determine the length of the marine growing season and, ultimately, the size attained by fish. Although the observed association between *MEP-2** genotype and age at maturity has been reported previously in Atlantic salmon (Jordan *et al.*, 1990; Morán *et al.*, 1998), and is likely to be widespread, the implications of these differences for conservation have not been fully explored.

The observed differences in *MEP-2** and mtDNA frequencies between angler caught fish and spawners strongly suggested that exploited fish are genetically, as well as phenotypically, distinct from spawners, hence the angling fishery exerts some artificial selection on the population. Moreover, if as suggested by some studies (Ballard & Kreitman, 1995; Hey, 1997), mitochondrial genes are also affected by natural selection, then the differential removal by anglers of certain haplotypes may also disrupt the adaptive architecture of these populations (Consuegra *et al.*, 2001, 2002). The selective exploitation of large fish may result in a lower fishery yield (Conover & Munch, 2002) and also in a loss of genetic diversity and an impoverished gene pool as have been observed in other fish species (Law, 2000, 2001; Kenchington, 2003).

Atlantic salmon populations subjected to the kind of seasonal exploitation exerted by anglers in Iberian rivers may be expected to change in several ways (Table VI). First, a shift towards delayed entry is likely to occur since timing of entry into the rivers is inherited (Hansen & Jonsson, 1991; Stewart *et al.*, 2002) and early running fish are exploited by the fishery while late running fish are not. Likewise, reductions in body size, sea-age and longevity are expected to occur, as these traits are also under genetic control in Atlantic salmon (Taylor, 1991; Rye & Refstie, 1995). Finally, genetic changes may also take place if spawners and angler caught fish differ with respect to *MEP-2** and mtDNA. Many of these predicted changes have been detected in Cantabrian populations (Table VI), indicating that angling for early running fish may induce detrimental genetic and phenotypic changes in Atlantic salmon populations.

The results suggest that early and late runs of Atlantic salmon constitute demographically and genetically distinct components of Iberian Atlantic salmon populations and that the selective exploitation of early running adults by angling may alter the adaptive architecture of these populations. It is recommended that the seasonal dimension is incorporated into the definition of operational conservation units for Atlantic salmon (Dodson *et al.*, 1998) and that rod and line fisheries are managed to reduce the risk of selective harvesting. Failure to do so may mean that well intentioned fishery regulations, such as fishing closures, may inadvertently compromise the same salmon populations they were designed to protect.

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