Anadromous and Catadromous
Fish Restoration Programmes: A
Time for Evaluation.

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THE USE OF GENETIC TAG IN THE EVALUATION OF FISH RESTORATION PROGRAMMES.

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ABSTRACT

The incidence of stocking programmes on natural populations of brown trout (*Salmo trutta*) in rivers of Navarra (Northern Spain) was investigated using *LDH-5** locus as a genetic maker. This locus is a useful marker because stock used to restocking these rivers are fixed for the LDH-5* 90 allele whereas this allele is not naturally present in wild populations of this area. Samples collected in stocked localities showed introgression rates ranging approximately from 0 to 50%. However these values, as well as the frequencies of *LDH-5* 90* allele, decreased after the stocking practices were interrupted in these localities. These results suggest a very low viability of stocked individuals in natural conditions which remain in the river few times and the apparent failure of stocking programmes in order to enhancement the natural populations.

I.INTRODUCTION

The fisheries represent an important international resource accounting for a substantial proportion of all food production and in many countries have also a considerable economic importance as recreational fisheries. The influence of human activities is thought to have contributed to a drastic decrease and extinction of a great number of populations and species of fish, especially of freshwater species. Human activities with the potential to alter the dynamic and structures of fish populations include not only overfishing itself but also habitat pollution (e.g., presence of heavy metals, acid rain), habitat alteration by man-made structures (e.g., dams which can alter the hydrographic regimen, prevent fish passage and destroy spawning sites) etc. (Sheridan, 1995).

Stocking programmes, that implies the massive release of hatchery reared fish, has been a universal common method for counteracting declines in size of fish populations and to improve the productivity of populations available to recreative or commercial fisheries. However, in last decade, along with the increase of awareness about the need of conservation of biological and genetic diversity, a general agreement has been reached on the danger of indiscriminate use of these practices. Possible impact of the release of reared fish can depend on wether they are infected with a disease organism, are exotic to the locality and how genetically different they are from the native populations. The genetic impacts of such releases can range from extinction (a complete displacement of indigenous populations), to various degrees of inbreeding with loss of genetic variation, to hybridization and at the opposite extreme no detectable introgression into native populations (Ferguson, 1990; Hindar et al. 1991; Crowl et al. ,1992; Heggberget et al., 1993; Vázquez et. al. 1993; Blanco et al. 1996).

At this moment, there are several reports describing basic principles for ameliorating harmful genetic effects of stocking activity. These principles include recommendations for effective numbers of parent fish used for producing stocking material and the preferred use of stocking material of local origin instead of exogenous hatchery fish, etc. (e.g., FAO/UNESP,1981; Hindar et al., 1991; ICES,1995; Cowx, 1994).

Brown trout, Salmo trutta, in one on the most valuables species that inhabit Spanish rivers. Existing populations, which are only open to recreational fishing, remain of considerable economic important, and attempts have been made over the past 30 years with the aim of enhancing natural populations or re-establishing extinguished ones through repopulations with foreign stocks imported mainly from central Europe (García Marín et al. 1991; Morán et al., 1991; Martínez et al. 1993; Blanco et al. 1996). However, there are few data about the consequences of these manipulations in relation to the viability of stocked individuals and their impact on native populations.

The aim of this work is the evaluation of effectiveness of the stocking policy and its impact on natural populations in rivers of Navarra (Northern Spain). These rivers have been stocked employing individuals from a stock (Oronoz-Mugaire Center) of German origin. The intensity of stocking and the stages used (0^+ and /or 1^+) differ in each river. After 1992, stocking practices were interrupted in the stocked localities studied in this study. The LDH-5* locus, which codes for the eye-specific lactate deshydrogenase in brown trout, was used as genetic diagnostic marker to distinguish between stocked and wild individuals.

II.MATERIALS AND METHOD

Samples of brown trout (*Salmo trutta*) were taken from 14 sites, including both Atlantic (6) and Mediterranean (8) drainage's, in Navarra (Northern Spain). Three sites were sampled once, eighth sites two times, two sites three times and one site four times between 1992 and 1995. Of those, samples from both unstocked and stocked locations were studied. Unstocked (5 sites including 13 samples) were caught in places where there are no records indicating hatchery stocking of any of these localities, however they are not isolated from possible migrations of hatchery fish planted from adjacent areas. Stocked (9 sites including 16 samples) were collected from places which have been stocked with individuals from Oronoz-Mugaire hatchery stock until 1992. The name and geographical location of these sites are shown in Tables 1, 2 and Figure 1. Also, a sample of hatchery stock used to restocking these rivers were analized.

Individuals from rivers were collected by electrofishing and stored at -40ºC until electrophoresis analysis. Eye extracts were assayed by horizontal starch (11%) gel electrophoresis to screening genetic variation at lactate deshydrogenase-5 locus (*LDH-5**). Tissue extracts preparation, electrophoresis techniques and staining procedures are described by Guyomard and Krieg 1983.

Allele frequencies were computed from each sample being considered as *100 the ancestral allele (Hamilton et al. 1989). Deviation of genotype proportions from those expected under Castle-Hardy-Weinberg equilibrium were quantified using F_{IS} (Wright, 1951) where F_{IS}= 1- (observed/ expected heterozygotes). The significance of F_{IS} was measured by $\chi^2 = n * (F_{IS})^2$ (Li and Horwitz, 1953).

III. RESULTS AND DISCUSSION.

Brown trout (Salmo trutta L) exhibits high levels of genetic variation and one of the largest populations subdivision of salmonids species (Ryman, 1983;Ferguson, 1989; Guyomard, 1989). Hamilton et al. (1989) examining the current distribution of the allelic frequencies at *LDH-5** locus suggest that brown trout populations can be subdivide in two groups or races: "ancestral race" fixed for the *LDH-5* 100* allele and the "modern race" characterized by the *LDH-5* 90* allele.

As has been reported previously, Spanish native population and hatchery stocks employed for their stocking appear to reflect these two distinct lineages. Stock used in the restocking of Spanish rivers seem to have a common origin and are fixed , or nearly fixed, for the **90* allele, whereas this allele is not naturally present in indigenous wild populations (García Marín et al., 1991; Morán et al., 1991; Martínez et al., 1993; Blanco et al., 1996).

Data obtained in this study clearly show differences in the allelic distribution at *LDH-5** locus between hatchery and wild samples (Tables 1 and 2). Hatchery stock is fixed for *90 allele, whereas this allele was detected in low frequency (between 0 and 0.168, with a mean frequency of 0.018) in samples caught in unstocked localities (Tables 1 and 2). In these samples (map code 1,3,7,10 and 12), one homozygous and six heterozygous individuals for the *90 allele were found out of 224 (3.12%) trout analized. The presence of these fish could be indicating movements of fish from adjacent stocked sites or the existence of a non-recorded stocking in these localities.

Also some differences can be observed between Atlantic and Mediterranean drainages (Tables 1 and 2). The alternative *LDH-5* 104* allele was found in 5 of 6 Atlantic localities sampled (frequency range between 0.022 and 0.25) and was absent in all Mediterranean samples (Tables 1 and 2). This geographical discontinuity of LDH-5* 104 allele also was anticipated in previous work (García Marín 1991; Cagigas 1994) and suggest the possible existence of, at least, two genetic lineages in Spanish natural population. In fact, differences between Spanish populations of Atlantic and Mediterranean drainages also were found in other loci (e.g., *CK-1, ADH, MDH-2*), having each drainage high frequencies at some alleles that are absent in the other (García Marín, 1991; Cagigas 1994 and unpublished results).

Samples caught in stocked localities showed higher frequencies of the *LDH-5* 90* allele than unstocked ones (Tables 1 and 2). A total of 66 individuals of stocked origin, including hybrids, were detected out 279 trout (23.66%) sampled in stocked sites (map codes 2, 4, 5, 6, 8, 9, 11, 13 and 14). The Zubiri (1992), Orbaizeta (1992) and Artabia (1993 and 1994) samples exhibited the highest **90* allele frequencies (between 0.345 and 0.75) whereas the 12 remaining stocked samples showed the *LDH-5*90* allele in low frequency (does not exceeding of 0.2) (Tables 1 and 2).

The presence of heterozygous individuals, aged 0^+, 1^+ and 2^+, appear to be indicating the existence in the wild of reproductive success between domesticated and native brown trout and represent an evidence of natural introgression. Previous studies on Spanish brown trout provide evidence of low incidence of stocking in river systems in contrast with the high introgression observed in lotic (lake and reservoirs) (Morán et al., 1991; García Marín et al., 1991; Martínez et al., 1993; Arias et al., 1995; Blanco et al.,1996).

Introgression rates (r), defined as the proportion of domesticated genes in a wild sample can be quantified in a randomly mating population as : $r = (f_s - f_n)/(f_d - f_n)$; where f_d is the frequency of a diagnostic allele in the hatchery stock; f_n is the frequency in the native population after stocking, and f_s the frequency in wild populations after stocking (Barbat-Leterrier et al., 1989).In this case using LDH-5* 90 allele as marker of introgression, $f_d = 1$ and assuming that $f_n = 0$ then $r = f_s$.

However, significant deviations from Hardy-Weinberg expectations, with a correspondingly extreme Wahlund effect were detected in most of the stocked samples where heterozygotes specimens were observed (Table 3). The excess of homozygotes **90/90* detected in these samples will be expected since domesticated fish (having **90/90* genotype) were released in that localities until 1992 and they can survive some time in the wild, together native individuals, before spawn and therefore samples caught in stocked localities will be an admixture of domesticated and native fish and no a random mating population. According to these findings, the frequency of *LDH-5* 90* allele estimated in stocked samples will be an overestimation of real values of introgression rates. Another approach to estimate a minimum of introgression rate can be made excluding **90/90* individuals to calculate f_s frequencies and use only *90/100 specimens which reflect the occurrence of a real introgression. Introgression rates calculated by both methods range between 0.559 (Max.) and 0.033 (min.) (Table 3). These values are similar than reported previously by Blanco et al. (1996) in populations of this area and lower than reported in other European populations (Barbat-Leterrier et al., 1989, Ferguson, 1989).

In other hand, a revision of data of Tables 1, 2 and 3 clearly shown that the frequency of *LDH-5* 90* allele and the values of introgression rates decrease in samples caught after 1992 when the stocking programmes was interrupted in these localities (Figure 2). These data, coupled with the low proportion of stocked individuals found in samples analyzed (14.51%), in spite of the large number of stocked fish released in this sites in the last twenty years, suggest that the majority of hatchery fish can not survive in natural conditions and few of them reach sexual maturity. These data shown an apparent failure of stocking programme in order to the enhancement natural populations and are very similar results to those reported by other workers. So, Beaudou et al. (1995) found that although the exact influence of stocking practices was difficult to evaluate, the restoration of a devastated Corsican river was mainly due to the brown trout populations of the tributaries

The apparently unsuccessful stocking in rivers of Navarra might be a fortunate circumstance from the viewpoint of conservation of genetic resources and show that was no justification for an indiscriminate stocking with foreign stock in these rivers where natural spawning occurs.

Therefore it could be unjustifiable to reject current stocking practices in a generalized way. Stocking may be inevitable for restoring populations at localities where the original populations have been extinct and useful in some conservation programmes in order to avoid that very small and endangered populations die out for purely demographic reasons. In these cases, before implementing a stocking programme it is important to identify the causes for the decline of numbers of individuals in the populations and to define alternative measures for elevating population sizes, in particular restoration of spawning grounds and regulation of fisheries.

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TABLE 1.- Allelic frequencies of *LDH-5** locus in samples of Atlantic drainages. (u= unstocked sites; s= stocked sites).

Map code	River sample	year	Frequency of LDH-5* alleles 104	100	90	%of90/100 individuals
1	Aritzakun(**u**)	1992	-----	0.905	0.050	10.00
		1993	0.022	0.978	-----	-----
2	Amaiur(s)	1992	0.022	0.841	0.137	9.09
		1993	-----	0.895	0.105	10.05
3	Zeberia(**u**)	1993	-----	1.000	-----	-----
		1995	-----	1.000	-----	-----
4	Erasun(s)	1994	0.222	0.778	-----	-----
		1995	0.250	0.750	-----	-----
5	Lesaka(s)	1994	0.063	0.750	0.187	12.50
6	Erasote(s)	1994	0.228	0.681	0.091	-----
	Hatchery stock	1992	-----	-----	1.000	-----

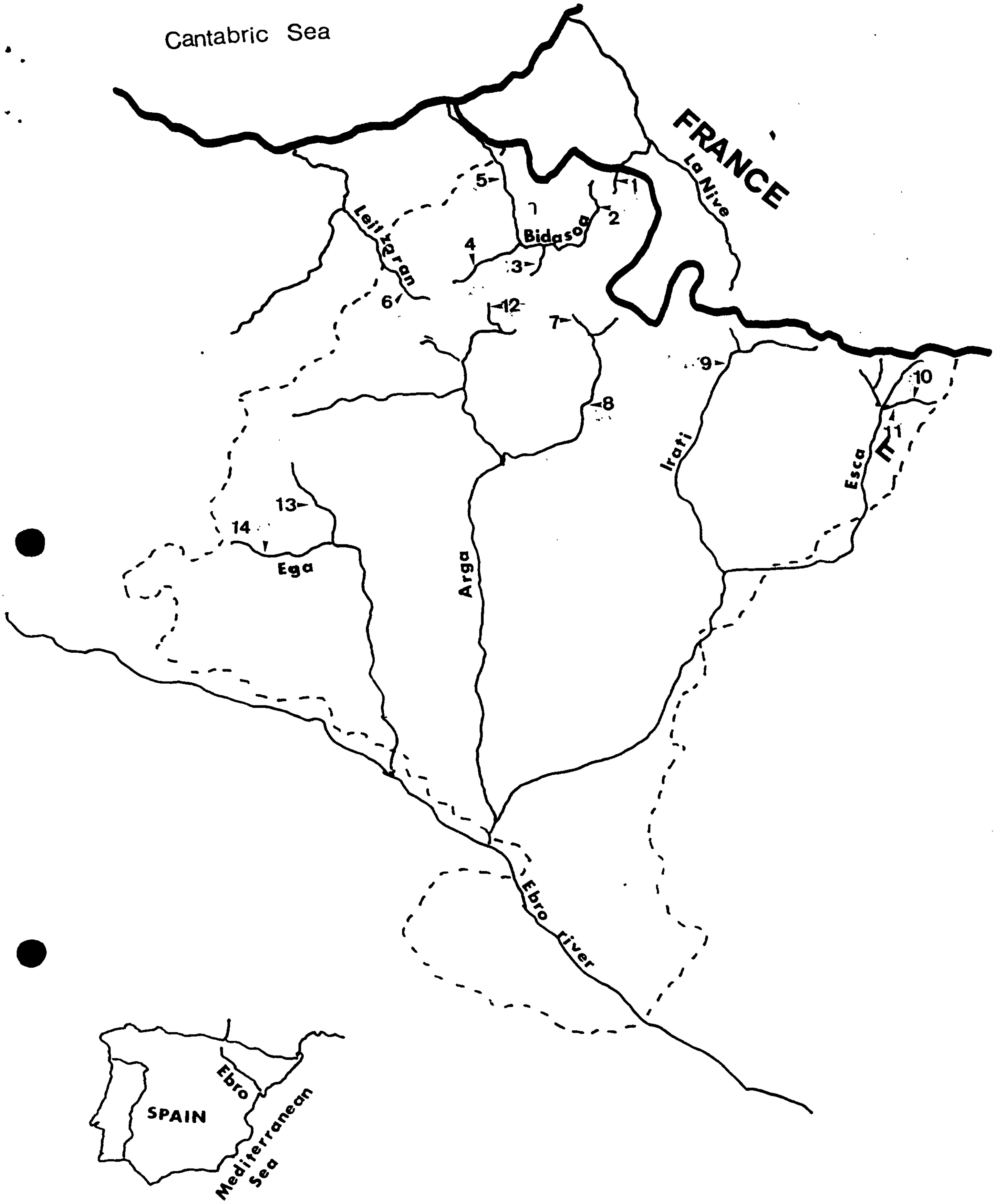
TABLE 2.- Allelic frequencies of *LDH-5** locus in samples of Mediterranean drainages. (u= unstocked sites; s= stocked sites)

Map code	River sample	Frequency of LDH-5*alleles year	100	90	%of90/100 individuals
7	Urcel (**u**)	1992	0.833	0.167	33.33
		1993	0.882	0.118	11.76
		1994	1.000	-----	-----
		1995	1.000	-----	-----
8	Zubiri (s)	1992	0.441	0.559	5.88
		1993	0.805	0.195	5.55
9	Orbaizeta (s)	1992	0.250	0.750	-----
		1993	0.800	0.200	-----
		1994	0.944	0.056	10.00
10	Belabarce-420(**u**)	1992	1.000	-----	-----
		1993	1.000	-----	-----
		1995	1.000	-----	-----
11	Belabarce-410(s)	1995	0.960	0.040	-----
12	Oriquieta (**u**)	1994	1.000	-----	-----
		1995	1.000	-----	-----
13	Artabia (s)	1993	0.531	0.469	31.25
		1994	0.652	0.348	26.08
14	Legaria (s)	1992	0.818	0.182	18.18
		1993	0.853	0.147	5.88

TABLE 3.- Values of FIS and maximun (Max) and minimun (min) introgression rates in samples with heterozygous (90/100) individuals

Map code	River sample	year	F_{IS}	Introgression rates Max	Min
2	Amaiur(s)	1992	-1.60***	0.137	0.050
		1993	-0.78**	0.105	0.055
5	Lesaka(s)	1994	-1.43**	0.187	0.071
8	Zudaire (s)	1992	-7.38***	0.559	0.062
		1993	-4.65***	0.195	0.033
9	Orbaiceta (s)	1994	-0.05	0.056	0.055
13	Artabia (s)	1993	-0.59*	0.469	0.227
		1994	-0.74**	0.348	0.166
14	Legaria (s)	1992	-0.63**	0.182	0.100
		1993	-3.30***	0.147	0.033
	mean values		-2.121 $\pm$0.735	0.238 $\pm$0.052	0.085 $\pm$0.020

* $P<0.05$; ** $P<0.01$; *** $P<0.001$.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Mediterranean Sea

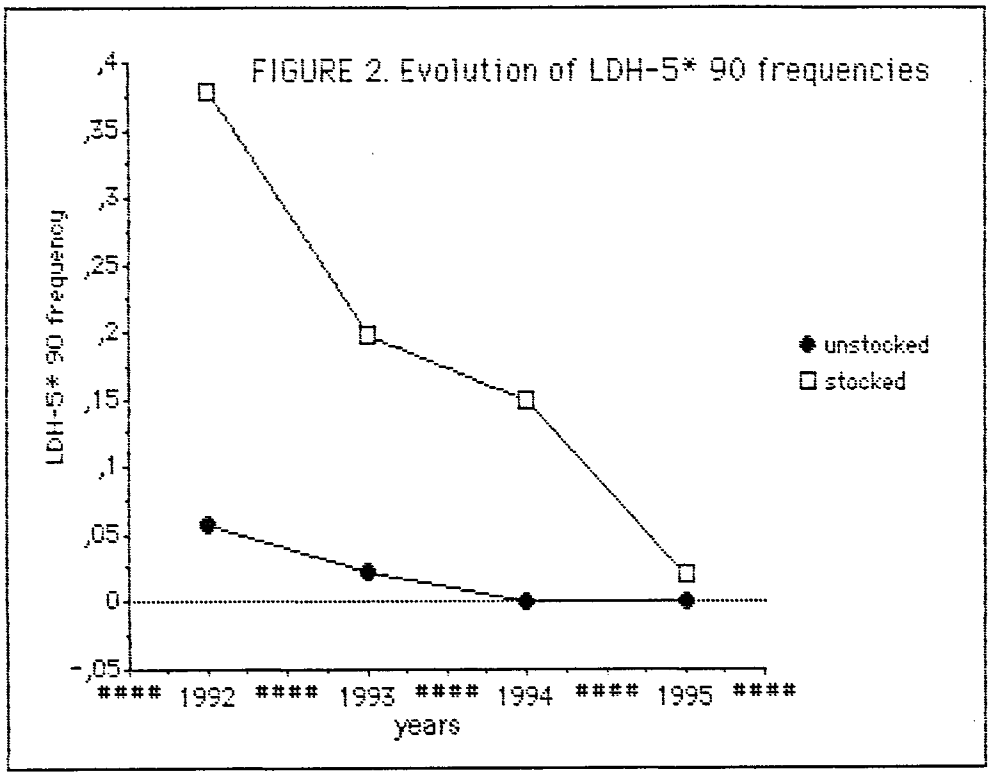
FIGURE 2. Evolution of LDH-5* 90 frequencies
LDH-5* 90 frequency
,4
,35
,3
,25
,2
,15
,1
,05
0
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1992 #### 1993 #### 1994 #### 1995
years
unstocked
stocked