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RELATIONSHIPS BETWEEN GENETIC VARIABILITY AND PRECOCIOUS MATURITY AND SMOLTING IN ATLANTIC SALMON.

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ABSTRACTS

In this work, we monitored in an Asturias hatchery (Northern Spain) a population of Atlantic salmon during its first year of life in fresh water. We had analyze the existence of genetic differences (using the six most polymorphics protein-coding-loci detected in Atlantic salmon: sAAT-4*, IDDH-1*, IDDH-2*, IDHP-3*, MDH-3,4* and mMEP-2) among different physiological groups (parr, precocious mature male and smolts).

Our results show that the smolt group has the greatest heterozygosity (30,12%) and mean number of heterozygous locus per individual (1.56). Parr group (non-mature and non-smolt fish) has significantly fewer values (21.43% and 1,25) than the other two groups, but precocious mature male (26,35% and 1.50) and smolts groups did not differ significantly ($t=0.49$; $P> 0.05$). In other hand, smolts group shows lower values (0,474) of frequency of mMEP-2*-100 allele than another two group.

These results suggest a positive relationship between the degree of heterozygosity and physiological changes in individuals under hatchery conditions. Due to the importance of this relationship a more intensive investigation on this subject is warranted.

Introduction

Atlantic salmon, *Salmo salar* L., is an anadromous specie which spawns in fresh water and migrate to the sea to feed. During its fresh water phase, a large phenotypic variability among individuals of the same population was observed. This interindividual variation is present in morphological and in physiological traits. So, in natural and cultured populations of Atlantic salmon, the existence of individuals with different growth rates results in a bimodal distribution of length and weight at the end of the first years of life (Thorpe et al. 1982, Metcalfe et al.1989, Nicieza et al 1991, Presa et al 1991). In terms of size, the "large" mode, (mainly females) is basically composed of individuals which smolt on the following spring and "smaller" mode contains remains parr and a variable fraction of precocious mature males (Ricker 1972, Thorpe and Moran 1980, Rowe and Thorpe, 1990, Presa et al 1991). It has been demonstrated that environmental conditions (photoperiod, temperature, etc.) play a role in the formation of this distribution, but the putative genetic bases of this intrapopulational variation remain unclear (Thorpe, 1986, Lundquist et al. 1986, Nicieza et al 1991).

Many recent publications describe associations between heterozygosity at protein-coding-loci and morphological variation within or among populations. In salmonids, as in other species, heterozygosity is positively associated with size, superior growth rate, superior viability, feeding rate or fecundity (Mitton and Grant, 1984; Liskauskas and Ferguson, 1990, 1991). Mitton and Koehn (1985) suggest that these associations exist because enzyme heterozygosity enhances physiological efficiency by decreasing the energetic cost of standard metabolism.

The aim of our work was to monitor a population of Atlantic salmon during its first year of life. At the end of the year the existence of 3 different subclasses of individuals was recognizable. Here, we analyze the possible genetic differences among groups by estimating their heterozygosity level loci using the 6 most polymorphic protein-coding-loci detected in Salmo salar in Spain: mMEP-2*, sIDHP-3*, sIDDH-1*, sIDDH-2*, sAAT-3* and sMDH-3,4* (Sánchez et al., 1991, 1993). The proposed null hypothesis is that the average heterozygosity is the same within each subgroup.

Material and methods:

Samples:

In December 1989, 15 female and 10 male Atlantic salmon, of two sea winters, were caught in Sella river (Asturias, Northern Spain) to be used as broodstocks to repopulate this river. Eggs from crosses between, at least seven females and five males, were collected to use in this experiment and raised in a hatchery. This minimum number (7 females and 5 males) represents an effective population size of 11.66 and, according to Nei et al. (1975), they would be expected to contain around of 90% the total genetic variability of the natural population. In September 1990, progenies from these eggs were randomly sampled and 200 individuals, previously anesthetized with Ms 222 (3-aminobenzoic acid ethyl ester), were individually tagged with a bar code using electric dermograph type Y.L.G. (electrical tattooing pen) and used for this study. From September to December, fork-length, weight precocios maturation and smolting was monthly monitored. Afterwards, the fish were sacrified for electrophoretical analysis and to correlate morphological and physiological parameters with their enzymatic genotype.

Electrophoresis:

Tissue samples of liver and muscle were analyzed by horizontal starch gel electrophoresis and scored for the 6 loci which show polymorphism in Asturias salmon populations: sMDH-3,4*, sIDHP-3*, mMEP-2*, sAAT-3*, sIDDH-1* and sIDDH-2*. The procedures followed those described by Sánchez et al., 1991 and Blanco et al., 1992.

Statistical analysis.

Student-t-test was used to test differences in length and weight between groups, as well as differences in mean heterozygosity.

The associations between number of heterozygous loci per fish and fish length, fish weight and growth rate in length and weight was determined using linear regression analysis.

All these tests are described in Sokal and Rholf, 1981.

Results and Discussion.

The September distribution of lengths approximated a normal distribution (fig. 1a).Fork lengths vary among 2.37 to 11.2 cm. with mean of 7.45 $\pm$ 0.11.

By December the distribution was distinctly bimodal (fig. 1b). If at this moment we also consider physiological criteria (precocious maturation and smoltification) we can divide the fish into 3 groups (fig. 1c):

1) Pre-smolts (33%): individuals from, in term of size, the larger modal group. These fish are mainly females and will smolt a little after their first year of life (in spring 1991).

2) Precocious mature individuals (5.6%): These individuals are situated between the two modal classes (see fig. 1b, c). All of them are males which have maturated at the end of their first year of life.

3) Parr (61.4%): individuals, which were all immature and would not smolt. Fig. 1c shows the population thus classified and Table 1 shows the mean values in length and weight in each group. The greatest differences in weight and length were found between pre-smolts group and the other one, Within groups from the small modal class (parr and precocious mature individuals) there are significant statistical differences in weight, but not in length (Table 1).

Table 2 shows different values of genetic diversity in each of the three different physiological classes of individuals. Significant heterogeneity of allele frequencies was found between group at 3 of the six enzymatic loci assayed (Table 2). Since some technical difficulties arisen to properly distinguish IDDH-1* and IDDH-2* genotipes in small individuals, results present for these loci should be relativized. Frequencies at mMEP-2* locus show great differences between groups. Pre-smolt individual has the lower values of mMEP-2*100 allele (Table 2). Recent studies provide evidences of an association between mMEP-2* variation and growth and spawning in Atlantic salmon (Jordan, et al. 1990). For this reason additional researches on this locus are needed.

On the other hand, pre-smolt group has the greatest heterozygosity and number of heterozygous loci per fish. Parr group has significantly fewer values than the other two groups ($t = 2.41$; $t=2.55$; $P < 0.05$), but mature and pre-smolt groups did not differ significantly ($t = 0.49$; $P>0.05$). (Table 2).

These results suggest a positive relationship between the degree of heterozygosity and physiological changes in individuals under hatchery conditions.

As we pointed at in Introduction, a positive association between heterozygosity at enzyme loci and growth rate has been reported in recent years in many animals and plant populations (see Mitton and Grant 1984 for a review). Mitton and Koehn (1985) suggested that this correlation is due to the fact that heterozygous individuals have increased metabolic efficiency compared to more homozygous individuals.

However, when we analyzed the growth rate for length (Gl) and weight (Gw) from September to December, the precocious mature male showed significant lower values (Gw=0.022 and Gl=0.015) than pre-smolts group (Gw=0.328 and Gl=0.112). However, both classes of individuals did not show significant differences in their level of heterozygosity (26.35% and 30.12% respectively, $t=0.49$).

These results do not invalidate the existence of a positive association between heterozygosity and the growth rate. As Saunders (1982) and Ferguson (1990) suggests, the precocious mature males may have a greater or similar growth rate than other individuals in the spring. By autumn, these individuals will initiate maturation deflecting their metabolic pathway and investing more energy towards gonads development and gametic production and, therefore, less energy to somatic tissue elaboration and so they will reduce their growth rate. In the period from September to December (when we analyzed our samples) they will show lower values of growth rate than other individuals with similar heterozygosity level. In this way, they will not be able to attain minimum size to smolt (Thorpe, 1977).

In summary our results suggest a positive relationship between the degree of heterozygosity and "performance" of the individuals under hatchery conditions. Due to the economic importance of these relationships we think that more intensive investigation of this subject is warranted.

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REFERENCES

Blanco G., et al, 1990. Superior developmental stability of heterozygotes at enzyme loci in Salmo salar. Aquaculture, **84**, 199-209.

Ferguson M.M., 1990. Enzyme heterozygosity and growth of rainbow trout reared at two ratios. Biol. J. Linn. Soc., **40**, 215-227.

Jordan W.C. et al. 1990. Genetic variation at the malic enzime-2 locus and age at maturity in sea-run Atlantic salmon *Salmo salar* L. J. Fish Aquat. Sci. **47**:1672-1677.

Liskauskas A.P., M.M. Ferguson, 1990. Enzyme heterozygosity and fecundity in a naturalized population of brook trout. Can.J.Fish. Aquat.Sci., **47**, 2010-2015.

Liskauskas A.P., M.M. Ferguson, 1991. Genetic variation and fitness: a test in a naturalized population of brook trout (Salvellinus fontinalis). Can.J.Fish. Aquat.Sci., **48**, 2152-2162.

Lundqvist H.,et al. 1986. Seawater adaptability in three different river stocks of Baltic salmon during smolting. Aquaculture, **52**, 219-229.

Metcalfe N.B. et al. 1989. Early social status and the development of life-history strategies in Atlantic salmon. Proc.R.Soc.Lond., **236**, 7-19.

Mitton J.B., M.C. Grant, 1984. Association among protein heterozygosity, growth rate and developmental homeostasis. Ann.Rev.Ecol.Syst., **15**, 479-499.

Mitton J.B., R.K. Koehn, 1985. Shell shape variation in the blue mussel and its association with enzyme heterozygosity. J.Exp. Mar. Biol. Ecol., **90**, 73-80.

Nei M., T. et al 1975. The bottleneck effect and genetic variability in populations. Evolution, **29**, 1-10

Nicieza A.G.et al. 1991. Development of length-bimodality and smolting in wild stocks of Atlantic salmon under different growth conditions. J.Fish Biol., **38**, 509-523.

Presa,P. et al. 1991.Analisis genético comparado de dos poblaciones de *Salmo salar* L de distinto origen geográfico. Proceeding de la Jornadas Luso-Españolas de Genética.

Ricker W.E., 1972. Hereditary and environmental factors affecting certain salmonid populations. In: Stocks concept in Pacific salmon.. H.R. MacMillan. British Columbia, Vancouver pp 27-160.

Rowe D.K. and Thorpe J.E. 1990. Suppresion of maturation in male Atlantic salmon parr by reduction in feeding growth during spring months. Aquaculture **86**: 291-313.

Sánchez J.A.et al. 1991. Allozyme variation in natural populations of Atlantic salmon in Asturias (northern Spain). Aquaculture, **93**, 291-298.

Sánchez J.A., et al.1993. Genetic status of Atlantic salmon (Salmo salar) in Asturian rivers (Northern Spain). In: Genetic Conservation of Salmonid Fishes,p.p. 219-225. J.Cloud & G.Thogart (eds.).Plenum Press.

Saunders R.L.et al.1982. Precocious sexual maturation and smoltification in male Atlantic salmon. Aquaculture, **28**, 211-229.

Sokal, R.R., F.J. Rholf, 1981. Biometry. W.H. Freeman and Co., San Francisco. 479.

Thorpe J.E., 1977. Bimodal distribution of length of Atlantic salmon under artificial rearing conditions. J.Fish Biol., **11**, 175-184.

Thorpe J.E., 1986. Age at first maturity in Atlantic salmon: freshwater period influences and conflicts with smolting. In: Salmonid Age at Maturity. Can.Spec.Publ.Aquat.Sci., **89**, 7-14.

Thorpe J.E., and R.I.G. Moran. 1980. Growth rate and smolting rate of progeny of male Atlantic salmon parr *Salmo salar*. J. Fish. Biol. **17**:451-459.

Thorpe J.E., et al. 1982. Bimodality of growth and smolting in Atlantic salmon. Aquaculture, **28**, 123-132.

TABLE 1.- Mean values of weight and length for each of four groups in which the December distribution was divided (see fig. 1c).

	Parr	Precocious mature males	Pre-smolts
Number of individuals	121	11	65
Weight (gr.)	3.94±0.08 a[1]	6.71±0.72b	16.32±0.42c
Length (cm.	7.07 + 0.21b[1]	8.26±0.24 b	11.29±0.09 c

(1) differences in mean values were tested using a Student- t- test, values with different letter are statistically different.

TABLE 2 .- Frequencies of the most common allele (100) and enzymatic variability in different physiological classes of individuals of Atlantic salmon.

Locus	Parr	Precocious mature males	Pre-smolts	Gtest[1]
MDH-3,4*	0.954	0.986	0.915	2.16
IDHP-3*	0.987	0.917	0.926	1.534
mMEP-2*	0.655	0.542	0.479	15.65**
AAt-4*	0.694	0.736	0.723	1.016
IDDH-1*	0.778	0.639	0.840	7.24*
IDDH-2*	0.785	0.722	0.904	9.382**
HET[2]	21.43%	26.35%	30.12%	
locus[3]	1.25	1.50	1.56	

(1) G-test for homogeneity of allele frequencies.
Average of heterozygosity (2) and mean number of heterozygous locus per individual (3) over six polymorphic loci scored.

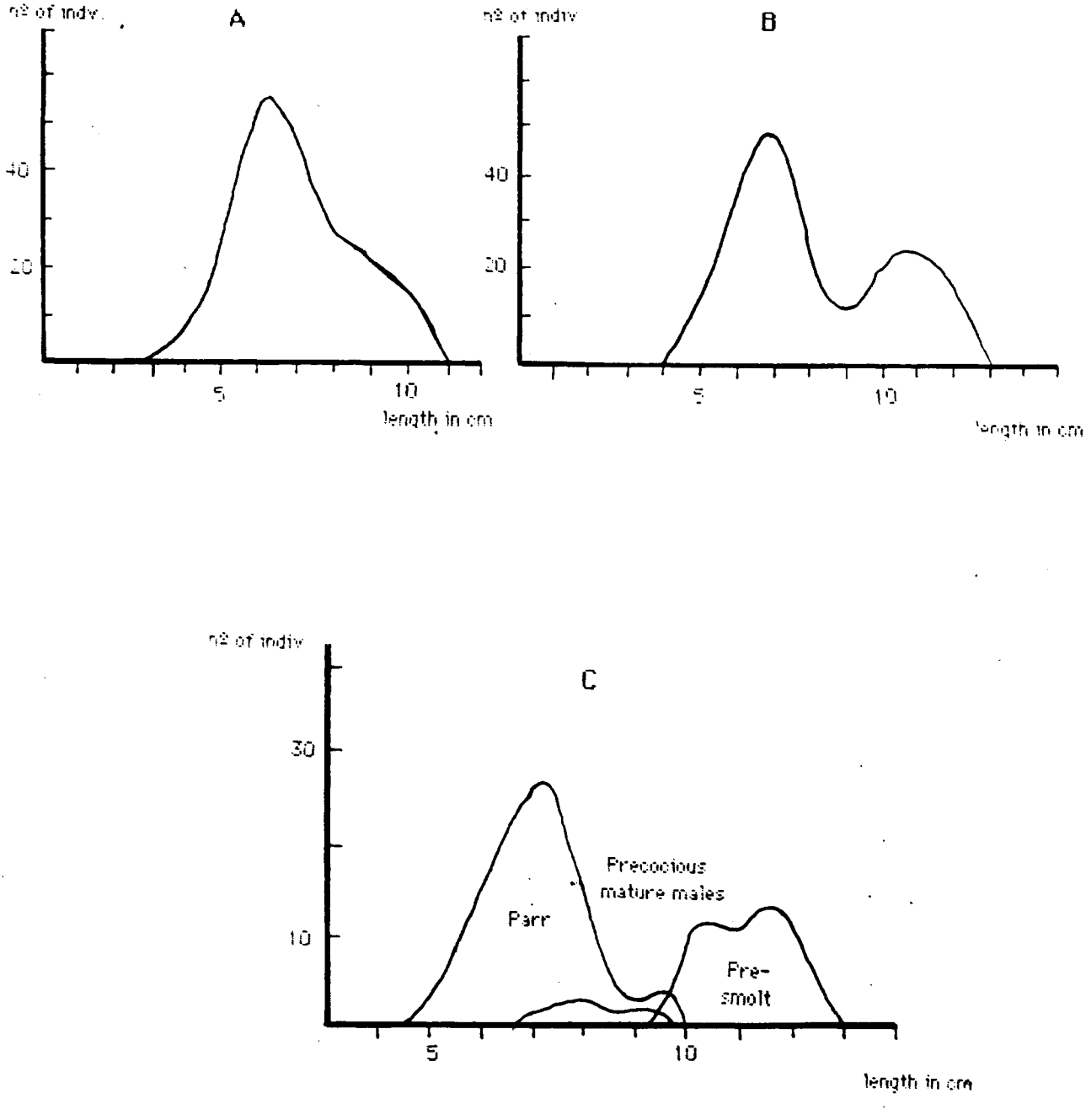

Figure 1: Distribution of fork-length
A) September; B) December; C) different groups of fish when December distribution were divided using physiological criteria.