

Stock transfers in Spanish brown trout populations: A long-term assessment

Jorge I. Izquierdo^{a,*}, Ana G. F. Castillo^{a,*}, Fernando Ayllon^a, Jeronimo de la Hoz^b & Eva Garcia-Vazquez^a

^a*Departamento de Biología Funcional, Universidad de Oviedo, C/ Julian Claveria s/n, 33006, Oviedo, Spain (e-mail: egv@fq.uniovi.es)*

^b*Servicio de Caza y Pesca, Principado de Asturias. Edificio Consejerías, C/ Coronel Aranda s/n, 33071, Oviedo, Spain*

Received 1 December 2004

Accepted 20 October 2005

Key words: foreign stocking, management policy, introgression, *Salmo trutta*

Synopsis

Stocking of fish from other populations has been commonly employed for enhancement of wild brown trout, *Salmo trutta*, populations in north Spain. Young hatchery reared brown trout of central European origin were introduced into some Asturian rivers every year since 1984. Based on variation at the isozyme locus *LDH-C1** and at the microsatellite locus BFRO 002, two genetic markers race-specific in *Salmo trutta*, we detected introgression of foreign genomes into native gene pools in some Spanish trout populations where only pure native individuals were present 10 years ago. We strongly suggest development of alternative management policies for conservation of Spanish natural brown trout populations without endangering the traditional recreational fisheries.

Introduction

In stock transfers, fish from one stock are introduced into a water body in a different geographic region inhabited by a different stock of that same species yet are still within their native range (Phillip et al. 1993). Although stocking efforts may affect the genetic integrity of local populations (Machordom et al. 1999, 2000), stock transfer was, and still is in some regions, a common management practice for wild salmonids. Stock transfers can also contribute to the loss of phylogeographic genetic patterns of a species (Ståhl 1987, Verspoor 1997), and potentially endanger local adaptation, crucial for salmonids (Taylor 1991).

Spain contains the southernmost European populations of brown trout, *Salmo trutta* L. All the

unstocked natural populations yet analysed (Moran et al. 1991) were fixed for the *LDH-C1*100* allele. Stock transfers into the Spanish rivers were common in the 20th century. Young hatchery-reared brown trout of central European origin, characterized by the *LDH-C1*90/90* genotype (Moran et al. 1991), have been introduced into some Asturian rivers every year since 1984 and they are still used for supplementing some brown trout populations. Genetic introgression of foreign genomes was reported for some regions (i.e. Machordom et al. 1999, 2000) with contrasting results of no introgression in other rivers or areas such as in the Cantabric coast (Moran et al. 1991).

Most of the largest and best-preserved Spanish salmonid populations inhabit the rivers of the central northern Spanish coast (Cantabric coast, region of Asturias; Figure 1), where they are exploited by recreational angling with rod and line. Their

* These two authors contributed equally to the article.

management is the responsibility of the Regional Administration, with the advice and collaboration of an Institution called Consejo Regional de la Pesca Fluvial (Regional Council of Freshwater Fisheries), where most angler associations are represented. Angling associations actively collaborate with river management, rearing stocks (both foreign and local) of brown trout for stocking.

The objective of this study was to determine long-term effects of foreign stocking by assessing the extent of introgression of foreign genomes into Asturian populations of wild brown trout.

Materials and methods

Populations analysed

We assessed brown trout populations inhabiting 13 Asturian rivers from the 7 main river drainages (Figure 1). They were supplemented with hatchery-reared foreign young in different years since 1984. Stocking effort varied among rivers, with a

maximum of 792000 foreign young brown trout released in the main stream of the River Narcea, as officially recorded by the Regional Administration (Table 1).

We carried out electrofishing in these 13 rivers between 1997 and 2003. Adult brown trout were anaesthetised and their adipose fin clipped and ethanol-preserved for genetic analysis.

There is no information about population trends before stock transfers, in the absence of catch records per river or other direct or indirect parameters.

Genetic analysis

The markers we chose for determining introgression of foreign genomes into Asturian local brown trout populations were the *LDH-C1** gene (Moran et al. 1991, McMeel et al. 2001) and the BFRO002 microsatellite locus (Susnik et al. 1997). After extracting DNA from adipose fin tissue following Estoup et al. (1996), the methodology was based on PCR amplification.

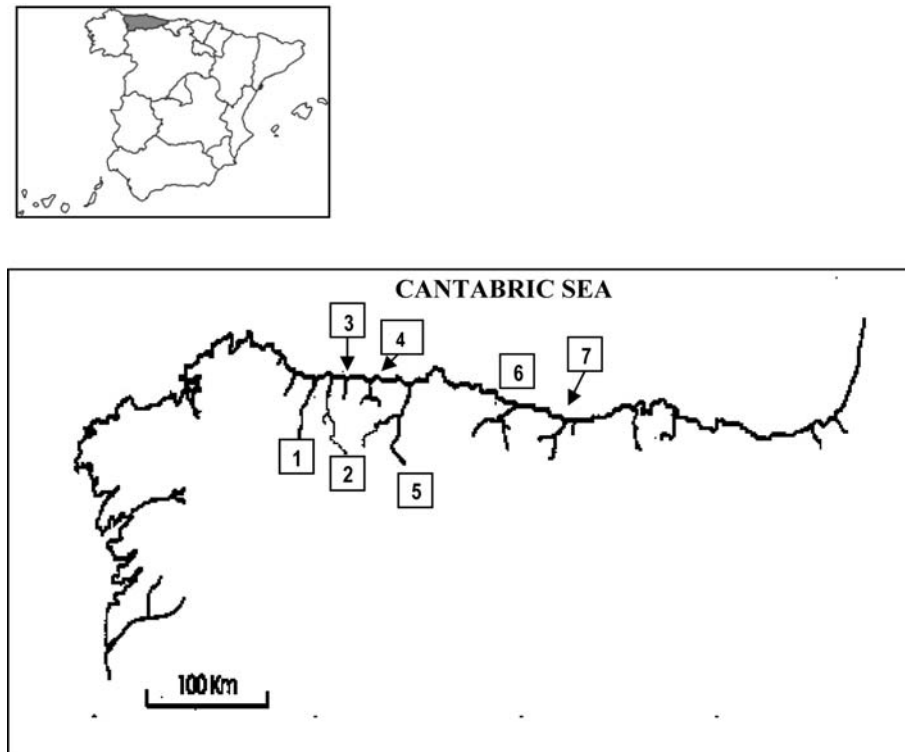


Figure 1. Map of the north Spanish study area: 1, River Eo; 2, River Porcia; 3, River Navia; 4, River Esva; 5, River Narcea-Nalon; 6, River Sella; 7, River Cares-Deva.

Table 1. Non-native brown trout, *Salmo trutta*, stocked in Asturian rivers: number (in thousands) of foreign alevins released in the studied rivers. Origin: Central Europe hatcheries.

River	Drainage	Foreign stocking
Cares	Cares	177
Sella	Sella	225
Piloña	Sella	573
Nalon	Narcea–Nalon	549
Nora	Narcea–Nalon	108
Caudal	Narcea–Nalon	783
Trubia	Narcea–Nalon	233
Narcea	Narcea–Nalon	792
Esva	Esva	103
Negro	Esva	42.5
Navia	Navia	133
Ibias	Navia	44
Porcia	Porcia	130

LDH-C1*

After PCR amplification of a fragment of the gene *LDH-C1**, we carried out digestion with *BsI* and visualization of the digestion fragments in an agarose gel, as described in McMeel et al. (2001). Briefly, digestion of a 440 bp region of the *LDH-C1** gene, using the enzyme *BsI*, generates two fragments of 360 and 80 bp for LDH-C1*90/90 individuals. Digestion of the DNA from *100/100 homozygotes produces a single uncut fragment of 440 bp, whereas heterozygote individuals generate all the three bands: 440, 360 and 80 bp long.

BFRO002

We chose the microsatellite locus BRFO002 as a marker based on characteristic alleles found in geographically remote areas (Susnik et al. 1997). Samples obtained in 2004 in some of the rivers above cited were genotyped for this microsatellite. To confirm its value for identification of foreign genes in the region studied, we analyzed samples from a domestic stock employed to supplement these rivers, as well as samples obtained in head tributaries of three basins (Cares, Navia, Esva and Porcia rivers), isolated from downstream areas due to impassable barriers to migration (natural obstacles, hydroelectric facilities) and where foreign stocking has never been carried out. We performed PCR amplifications on reaction mixtures containing 10 nM Tris–HCl pH 8.8, 1.5 mM

MgCl₂, 50 mM KCl, 0.1% Triton X-100, 0.35 μM of either fluorescently labelled or non-labelled primers, 0.5 units of DNA Taq Polymerase (Promega, Madison, WI), 250 μM of each dNTP and approximately 50 ng of extracted brown trout DNA template, in a final volume of 20 μl. Size determination of the labeled PCR products was performed using an ABI 3100 automatic DNA Sequencer and the GENESCAN V. 3.7 software at the DNA Sequencing Unit of the University of Oviedo (Spain).

Results

Introgression of foreign genomes in Asturian brown trout populations

LDH-C1* genotypes found for the brown trout sampled from our 13 study rivers are summarized in Table 2. As this marker is absolute, foreign individuals can be identified by their *90/90 genotype, heterozygotes being the product of crosses of native and foreign trout. The presence of foreign adults was confirmed in five rivers (Sella, Nalon, Nora, Caudal and Esva). Evidence of reproduction of foreign individuals with native ones (heterozygotes) existed in four of these five rivers (Sella, Nalon, Nora, and Caudal) and also in the River Navia, where pure foreign adult were not found. In the

Table 2. LDH-C1* genotypes for the brown trout populations analysed (N = 1286 individuals).

River	Genotypes		
	*100/100	*90/100	*90/90
Cares	50	0	0
Sella	177	2	5
Piloña	50	0	0
Nalon (upstream)	227	0	0
Nalon (Caleao)	37	15	0
Nalon (Rioseco reservoir)	0	0	50
Nora	23	4	8
Caudal	117	28	8
Trubia	50	0	0
Narcea	85	0	0
Esva	98	0	2
Negro	50	0	0
Navia	77	23	0
Ibias	50	0	0
Porcia	50	0	0

Table 3. (a) BRFO002 allele frequencies found in one domestic stock employed to supplement native brown trout populations in the area ($N=50$), and in inaccessible never stocked wild populations sampled in 2004 ($N=280$ individuals). (b) Frequency of the allele 118 found in individuals sampled in 2004, from river areas where foreign stocking had been carried out ($N=268$).

(a)		Alleles					
Sample	N	118	120	122	124	126	128
Domestic	50	0.990	0	0	0.010	0	0
Upstream Porcia	30	0	0.067	0.367	0.317	0.250	0
Upstream Navia	86	0	0	0.424	0.262	0.307	0.064
Upstream Esva	120	0	0	0.558	0.096	0.346	0
Upstream Cares	44	0	0	0.284	0.511	0.205	0
(b)		118 allele frequency					
River	N						
Cares	42	0					
Sella	54	0					
Piloña	8	0					
Esva	88	0.011					
Negro	45	0					
Porcia	31	0					

River Nalon, foreign individuals were spatially segregated from the rest, being restricted to a reservoir area with very low water flow. Heterozygotes were present in a stream near the reservoir. Native brown trout was the only variant found upstream, in head river tributaries. The global *90 allele frequency was 0.175 for this river. In the Sella and Esva rivers the frequency of foreign adults or heterozygotes was very low, *90 allele frequency being 0.032 and 0.02, respectively. In the Navia, Caudal and Nora rivers, however, the *90 allele frequencies were much higher: 0.115, 0.144 and 0.286, respectively. In the other seven rivers (Cares, Piloña, Trubia, Narcea, Negro, Ibias and Porcia) only native brown trout was found despite high stocking effort in some cases (Narcea and Piloña rivers, where more than 500 000 foreign trout had been released). There was no statistical association between stocking effort and introgression of foreign genomes ($r=0.109$, 11 d.f., n.s.).

With respect to the marker BRFO002 (Table 3a), we found six different alleles in the samples we analyzed. In the domestic stock only two alleles appeared, 118 (the most frequent) and 124 (one unique allele in a heterozygote). In the samples obtained in inaccessible sites never stocked (upstream Navia, Porcia and Esva rivers), we found five different alleles: 120, 122, 124, 126 and 128. Therefore the allele 118 can be considered a marker allele for identification of the domestic stock. This allele was almost absent in trout sam-

pled from areas where domestic alevins had been released (Table 3b), indicating low level of introgression of domestic genomes in the region. In the River Esva some introgression (two individuals %) were detected employing the marker *LDH-C1**. Similar introgression was also detected with this marker (2.2% individuals were heterozygotes for the allele 118). In the rest of the rivers sampled in 2004 introgression was not detected employing this microsatellite locus as a marker. This is consistent with the results obtained for the marker *LDH-C1** (Table 2).

Discussion

Our results show introgression of foreign genomes into north Spanish populations of brown trout. For the six rivers where two independent genetic markers were analysed (Cares, Sella, Piloña, Esva, Negro and Porcia rivers), results were consistent for both markers; around 1% introgression was detected combining the six rivers (0.7% employing the microsatellite BRFO002, 1.8% with the locus *LDH-C1**). In the whole study, genetic introgression of central European genomes was detected in six out of 13 rivers considered. The frequency of foreign alleles was higher than 0.1 in four rivers. This means 10% foreign variants. Heterozygotes were present in five rivers, indicating a relevant contribution of foreign trout to natural spawning.

At the beginning of the 1990's, stock transfers were reported to have negligible effects on the genetic pool of Asturian populations (Moran et al. 1991). Ten years later, introgression is confirmed in more than one quarter of the rivers considered, with no association to stocking effort. Introgression of central European genes into Iberian trout populations has also been reported for other areas (García-Marín et al. 1998, Machordom et al. 2000). On the other hand, variable levels of hatchery trout introgression depending on particular river conditions seem to be a characteristic for this species (i.e. Ruzzante et al. 2001, Hansen 2002). These results emphasize the need for envisaging alternative by-river management policies instead of relying in a combination of fishing+stocking (Allendorf et al. 1987, Almodovar & Nicola 2004).

There is an urgent need for dissemination of these results amongst users of this natural resource, including anglers, administrators, managers, tourists and also conservationist associations and the general public. Public dissemination of scientific results has proved to be an excellent tool for designing conservative management policies in other cases of exploited wild populations (Odum 1993). Involvement of users in management ensures the implementation of conservative practices if they are supported by a critical mass of citizens (Stake 1995). This social aspect is most probably the next step needed for the conservation of Spanish salmonids.

Acknowledgements

We are grateful to Ivan Gonzalez Pola (Universidad de Oviedo) for his help in laboratory analysis. This work was supported by the Spanish Project MCYT REN2003-00303 and by the Principado de Asturias (Spain). E. Garcia-Vazquez received a Grant from the Spanish Government (PR004-0084).

References

Allendorf, F.W., N. Ryman & F.M. Utter. 1987. Genetics and fishery management: past, present, and future. pp. 1–19. *In*: N. Ryman & F.M. Utter, (eds.), Population Genetics and Fishery Management, University of Washington Press, Seattle.

- Almodóvar, A. & G.G. Nicola. 2004. Angling impact on conservation of Spanish stream-dwelling brown trout *Salmo trutta*. *Fisheries Management and Ecology* 11: 173–182.
- Estoup, A., C.R. Largiader, E. Perrot & D. Chourrout. 1996. Rapid one-tube extraction for reliable PCR detection of fish polymorphic markers and transgenes. *Molecular Marine Biology and Biotechnology* 5: 295–298.
- García-Marín, J.L., N. Sanz & C. Pla. 1998. Proportions of native and introduced brown trout in adjacent fished and unfished Spanish rivers. *Conservation Biology* 12: 313–319.
- Hansen, M.M. 2002. Estimating the long-term effects of stocking domesticated trout into wild brown trout (*Salmo trutta*) populations: An approach using microsatellite DNA analysis of historical and contemporary samples. *Molecular Ecology* 11: 1003–1015.
- Machordom, A., J.L. García-Marín, N. Sanz, A. Almodovar & C. Pla. 1999. Allozyme diversity in brown trout (*Salmo trutta*) from Central Spain: Genetic consequences of restocking. *Freshwater Biology* 41: 707–717.
- Machordom, A., J. Suarez, A. Almodovar & J.M. Bautista. 2000. Mitochondrial haplotype variation and phylogeography of Iberian brown trout populations. *Molecular Ecology* 9: 1324–1338.
- McMeel, O.M., E.M. Hoey & A. Ferguson. 2001. Partial nucleotide sequences, and routine typing by polymerase chain reaction-restriction fragment length polymorphism, of the brown trout (*Salmo trutta*) lactate dehydrogenase, LDH-C1*90 and *100 alleles. *Molecular Ecology* 10: 29–34.
- Morán, P., A.M. Pendas, E. Garcia-Vazquez & J.I. Izquierdo. 1991. Failure of a stocking policy of hatchery reared brown trout, *Salmo trutta* L., in Asturias, Spain, detected using LDH-5* as a genetic marker. *Journal of Fish Biology* 39(A):117–121.
- Odum, E. 1993. *Ecology and Our Endangered Life-Support Systems*. 2nd edition. Sinauer Associates, Inc, Sunderland, Massachusetts. 301 pp.
- Philipp, D.P., J.M. Epifanio & M.J. Jennings. 1993. Conservation genetics and current stocking practices: Are they compatible? *Fisheries* 18: 14–16.
- Ruzzante, D.E., M.M. Hansen & D. Meldrup. 2001. Distribution of individual inbreeding coefficients, relatedness and influence of stocking on native anadromous brown trout (*Salmo trutta*) population structure. *Molecular Ecology* 10: 2107–2128.
- Ståhl, G. 1987. Genetic Population Structure of Atlantic Salmon. pp. 12–140. *In*: N. Ryman & F. Utter, (eds.), Population Genetics and Fishery Management, University of Washington Press, Seattle.
- Stake, R.E. 1995. *The Art of Case Study Research*. Sage Publications, Thousand Oaks. 192 pp.
- Susnik, S., A. Snoj, J. Pohar & P. Dovc. 1997. The microsatellite marker (BRFO 002) characteristic for different geographically remote brown trout, *Salmo trutta* L., populations. *Animal Genetics* 28: 70–383.
- Taylor, E.B. 1991. A review of local adaptation in Salmonidae, with particular reference to Pacific and Atlantic salmon. *Aquaculture* 98: 185–207.
- Verspoor, E. 1997. Genetic diversity among Atlantic salmon (*Salmo salar* L.) populations. *ICES Journal of Marine Science* 54: 965–973.