Forensic Science International 272 (2017) 37–40

Contents lists available at ScienceDirect

Forensic Science International

journal homepage: www.elsevier.com/locate/forsciint

Forensic assignment to geographic origin, a useful tool in seafood fraud control

J.L. Horreo[a,*], G. Machado-Schiaffino[b], E. García-Vázquez[c]

[a] Department of Biodiversity and Ecologic Restoration, Instituto Pirenaico de Ecología (IPE-CSIC), Avda. Nuestra Señora de la Victoria 16, 22700 Jaca, Spain
[b] Zoology and Evolutionary Biology, Department of Biology, University of Konstanz, Universitatstrasse 10, 78467 Konstanz, Germany
[c] Department of Functional Biology, University of Oviedo, C/Julián Clavería s/n, 33006 Oviedo, Spain

ARTICLE INFO

Article history:
Received 4 October 2016
Received in revised form 5 December 2016
Accepted 2 January 2017
Available online 10 January 2017

Keywords:
Atlantic salmon
Court
Evidence
Genetic identification
Salmo salar
Trial

ABSTRACT

Seafood fraud is an economically motivated and widely spread problem encompassing drastic consequences in both public health and species conservation. In Northern Spain, only the first Atlantic salmon (*Salmo salar*) catch of the angling season (named *Campanu*) can be sold. In the year 2011, an angler denounced it on regional Court claiming that the *Campanu* (which was sold in 6000€) was fraudulent because it had been caught from another river than the fisherman ("the seller") stated. Here, we report the first judicial case of application of geographical genetic assignment in a fish species in Spain. In order to accomplish this, genetic assignments to their rivers of origin of the *Campanu* and another three following salmon catches of the angling season of the year 2011 were performed. A panel of eight microsatellite loci together with a comprehensive genetic baseline of the rivers of the region were employed. Results showed that the *Campanu* was the only case in which genetic assignment and fisherman declaration of the river of origin did not match. The methodology here employed showed to be very useful as a reinforcement of other evidences contributing to fight against seafood fraud in Courts.

1. Introduction

Seafood fraud is an important problem for many reasons and, unfortunately, has becoming widely spread in the last decades, especially in fish [1]. Economic reasons underlie seafood fraud in most cases: species of low commercial values are sold for others more expensive in order to increase benefits [2]. However, this economically motivated adulteration can encompass drastic consequences affecting public health [3] and species conservation [4]. For all these reasons, seafood forensics helps to combat mislabelling and fraud, being DNA-based technology essential on it [5]. DNA is often employed for species ascertainment [6]. Less frequently, the identification of the source population is done from DNA because the fraud is based on selling individuals from one population as if they were from other, generally more expensive or appreciated [7]. In these cases, specific tools must be developed.

A very special type of seafood is highly valued wild fish. Wild Atlantic salmon (*Salmo salar*) is an iconic species, with its southernmost European populations (Asturias, Northern Spain) in marked decline [8]. For this reason these populations are not commercially exploited. Strict quotas per angler and season have been enforced by law for sport angling and are being applied for the last decades (Law 6/2002 of the Regional Government of the Principality of Asturias). Other rules included in this law are: mandatory register of individual catches, a short period of angling – between March and July –, bans (two days a week) during the fishing season, and the prohibition of selling wild salmon from the rivers, with only one exception: the first catch of each angling season, per river. This exceptional individual is locally called *Campanu*, derived from the Spanish word *Campana* (bell) as in the past church bells rang to announce the first Atlantic salmon catch of the year in Asturias [9]. It is sold in a public auction reaching extraordinary prices: up to 18,000€ were paid for a 4.4 kg *Campanu* in 2007. Bidding is independent in the different rivers of the region where angling is allowed (from east to west: Cares, Sella, Narcea, Esva and Eo rivers; Fig. 1), and the auction generally reaches a higher *Campanu* value in River Narcea, especially when it is also the first catch of all the rivers.

In the year 2011, a 5.8 kg *Campanu* was registered as caught from River Narcea early in the morning of the season opening day (*Campanu* 2011 hereafter), and sold in the auction for 6000€. Immediately, another angler denounced it on the regional Court claiming that such *Campanu* was fraudulent because it had been caught from another river (River Esva). Thus, the salmon caught in

* Corresponding author.
E-mail address: horreojose@gmail.com (J.L. Horreo).

http://dx.doi.org/10.1016/j.forsciint.2017.01.003
0379-0738/

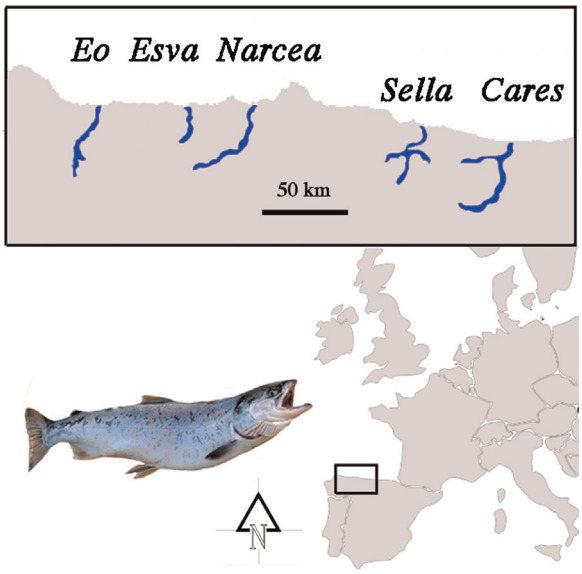

Fig. 1. Map showing the region of Asturias (Northern Spain), and the rivers included in the genetic baseline employed for the forensic assignment of *Campanu* 2011 to a river: Eo, Esva, Narcea, Sella and Cares. Atlantic salmon photograph credits: the authors taken it from http://www.publicdomainpictures.net.

river Narcea by the accuser deserved to be considered as the new Campanu 2011 for that river.

The Court (Juzgado de Primera Instancia e Instrucción n.1 de Grado, Asturias) was provided with several kinds of evidences for the trial, including witnesses' declarations. They considered the genetic identification of the *Campanu* 2011 natal river would be useful as a complementary proof and contacted the Laboratory of Genetic Resources of the University of Oviedo (Spain) asking for river assignment from genetic analysis. The mentioned laboratory had worked in genetic assignment of Atlantic salmon to natal rivers in the whole Atlantic Arc [10] and had access to a complete genetic baseline of the populations inhabiting the rivers of the region [8]. *Campanu* 2011 and the three next salmon catches of the same day in the region were assigned to the rivers of the mentioned baseline using eight microsatellite loci and GENECLASS2 software [11]. The same methodology had been already employed in a court processes in Norway for forensic assignation of escapees to Norwegian Atlantic salmon farms [12]. Results were reported in the trial of *Campanu* 2011 fraud.

2. Materials and methods

2.1. Sample collection

Agents of the Service of Protection of Nature SEPRONA (in charge of environmental surveillance in Spain) obtained Atlantic salmon scales (n = 3) and adipose fin tissue (n = 3 biopsies) from *Campanu* 2011. They were in charge of custody of the samples and brought them to the laboratory of Genetics of natural Resources (University of Oviedo). The same procedure was also done with the second, the third and the fourth salmon catch registered in the region the same fishing season of 2011. These samples were caught in the River Narcea (sample N1) and in the River Esva (samples E1 and E2).

2.2. The reference baseline

The reference baseline was composed by the genetic information of 90–100 individuals per river (years 2002 and 2007) of the Eo, Esva, Narcea, Sella and Cares rivers (total of 489 samples, including both juveniles and adults). A total of eight microsatellite loci were genotyped for each individual sample. This baseline has demonstrated to be useful for river assignment and as such has been previously employed for determining the population structuring of the species in the region [13], and for estimating the effective population size of each river [8].

2.3. DNA extraction and PCR amplification

DNA extraction was done for duplicate from each sample, independently and in a cabin with laminar flow to avoid contaminations. Extractions were done using Chelex resin (Biorad) protocol as in Horreo et al. [8]. Both negative and positive controls were included. Small pieces of salmon tissue were introduced in a 1.5 ml Eppendorf tube with 500 μl of 10% Chelex100 and 7.5 μl K-proteinase (20 mg/ml). It was incubated under agitation at 55 °C for 1.5 h followed by 20 min at 100 °C. Resulting DNA was stored frozen at −20 °C in the Laboratory of Natural Resources of the University of Oviedo (Spain).

For the genetic assignment of samples to origin river, the same eight microsatellite loci of the genetic baseline were amplified as described in [8] protocols: SSsp2210, SSspG7, SSsp1605, Ssa197, Ssa202, SSOSL417, SSOSL85 and SSOSL311. Note that these markers had previously demonstrated to be sufficiently informative for parentage assignment in these populations [14].

2.4. Genotyping and genetic assignment

Allele sizes were determined with an automatic sequencer (ABI Prism 3130, Applied Biosystems) and the GeneMapper v3.5 software (Applied Biosystems) in the Unit of DNA Analysis of the University of Oviedo. Several samples of known genotype were run at the same time as positive controls for standardising sizes and avoiding genotyping errors. The allele frequency of each *Campanu* 2011 allele was calculated for the genetic baseline with the software GENETIX [15].

Following Ref. [12], genetic assignments of the salmon samples to the baseline were done with the GENECLASS2 software [11]. This software has been widely used for assignments to populations or stocks of Atlantic salmon and other species, as for example individuals present in sea waters [16], recolonizing new river areas [17] or entering in protected spaces [18]. GENECLASS2 provides sample assignment probabilities of belonging to each of the baseline populations, and does not assume that all potential source populations have been sampled, thus allowing rejecting unknown individuals from baseline populations if those were potentially incomplete. To do it, the probability computation of Monte-Carlo resampling of [19] was enabled with 1,000,000 simulated replicates and 0.01 of type I error (alpha). Calculations were performed under two different computational criteria for ensuring results: R&M: [20] and B&L: [21] (see Ref. [22] for complete details about them). In addition, likelihood ratios of the individuals for belonging to each river were estimated as −log(L) under a frequencies-based method [23] with a frequency for missing allele of 0.01.

3. Results

All the six *Campanu* 2011 samples analysed (3 scales and 3 tissue samples) had exactly the same genotype (Table 1) with clear electropherogram peaks. It was homozygote at the SSspG7 locus (allele 144) and heterozygote at all the other loci. The other three individuals (Table 1) had also high heterozygosity levels. The four Atlantic salmon individuals analysed exhibited unique genotypes when the eight microsatellites loci are considered.