



Full length article

Economy matters: A study of mislabeling in salmon products from two regions, Alaska and Canada (Northwest of America) and Asturias (Northwest of Spain)



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ARTICLE INFO

Handled by George A. Rose

Keywords:

Socioeconomic perspective

Salmon

Trout

Mislabeling

COI

ABSTRACT

Mislabeling of seafood species has negative economic, social and ecological consequences, from consumer losses due to fraudulent exchange, undermining consumer awareness, to hiding illegal and unreported catches. Salmonids are no exception. They are an important part of the culture and economy of many countries in the northern hemisphere, and identifying possible causes of salmon mislabeling is of great interest, even more so where wild species and species from aquaculture are consumed. Here different types of commercial unrecognizable salmonid products (111 in total) from Asturias in Northwest Spain (Atlantic Ocean), and Alaska and Vancouver Island in Northwest America (Pacific Ocean) were analyzed by DNA Barcoding. The Spanish and Northwest American samples were mislabeled 6% and 23.8% respectively. Species substitutions were respectively wild-farmed and wild-wild, substitute species being cheaper. Economic reasons and social preference of wild over farmed products seem to be the main drivers in the exchanges detected in this study. Enhancing controls over the unrecognizable products to prevent this type of fraud is essential and strongly recommended.

1. Introduction

Fish mislabeling results in a series of varied negative consequences that have been well summarized by Jacquet and Pauly (2008). The economic advantages for the defrauders that obtain profits by selling cheaper species for more expensive fish represent losses in duties and import taxes for governments, as well as inadvertent economic losses for the consumers that buy an unwanted product at expensive prices. At the social level, fraud undermines efforts of sustainable fisheries and aquaculture, and consumer's eco-awareness. For example, if the fish are sustainably caught as it happens for instance for South African hake (Marine Stewardship Council: certified sustainable seafood 2004; as <https://www.msc.org/>) but are sold abroad under a wrong label (Garcia-Vazquez et al., 2011; Muñoz-Colmenero et al., 2015), the enormous effort of stakeholders for achieving sustainability cannot be recognized by the unaware foreign consumer. Other consequences are ecological. Mislabeling may hide cases of illegal fisheries, for example: the complex case of Pacific rockfish (Logan et al., 2008), endangered

angel shark mixed with other sharks (Ardura et al., 2011), mislabeling of cod products in China markets (Xiong et al., 2016), the use of *Gadus chalcogrammus* as substitute of *Merluccius merluccius* (Ferrito et al., 2016), and others.

One case of special importance from a social point of view are salmonids. Wild salmonids are an essential part of traditional cultures in many societies of the northern Hemisphere, both in North America (Finney et al., 2000; Raby et al., 2012) and Europe (Briton, 2014; Valiente et al., 2011) as well as key economic resources especially along the North Pacific American coast. On the other hand, some species such as the Pacific rainbow trout *Oncorhynchus mykiss* and the Atlantic salmon *Salmo salar* are widely cultivated. Wild and farmed salmonids are differently appreciated by consumers worldwide, for example, Verbeke et al. (2007) found that consumers slightly preferred wild over farmed fish on the attributes of taste, health and nutritious value. Consumer's perception about which seafood type conveys the highest quality clearly favors fish and shellfish harvested from the wild, with 53% of consumers preferring wild-caught seafood in the USA (O'Dierno

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<http://dx.doi.org/10.1016/j.fishres.2017.07.012>

Received 20 June 2016; Received in revised form 7 July 2017; Accepted 13 July 2017
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Table 1

Examples of mislabeling reported in salmonids. Percent of mislabeling reported, and percent of *Salmo salar* and *Oncorhynchus* (any species) declared in the labels and found from DNA in each study.

Region	N	Mislabeling detected		Label species		Real species		Reference
				<i>Salmo salar</i>	<i>Oncorhynchus</i> spp.	<i>Salmo salar</i>	<i>Oncorhynchus</i> spp.	
South Africa	12	8.3%		75%	25%	66.7%	33.3%	Cawthorn et al. (2012)
Northeastern America	9	0		55.6%	44.4%	55.6%	44.4%	Wong and Hanner (2008)
USA	384	7.3%		5%	95%	9.6%	90.4%	Warner et al. (2013)
Washington State	99	20.2%		0	100	11.3%	88.7%	Cline (2012)

et al., 2006). Wild salmon is preferred at least in part due to the general belief that farmed fish contain more mercury than wild fish (average 68.5% European consumers; Pieniak et al., 2013).

The use of DNA is required to identify the species when fish are sold in pieces or processed in any way so that their morphological characteristics cannot be recognized (Muñoz-Colmenero et al., 2015). Several studies applying DNA Barcoding have reported mislabeling in salmonids (e.g.: Pardo et al., 2016; Cawthorn et al., 2012; Cline, 2012; Warner et al., 2013; Wong and Hanner, 2008). In these studies (Table 1) the species substituted with is often farmed salmon for wild salmon (e.g., Atlantic salmon sold instead of king salmon *Oncorhynchus tshawytscha*; Cline, 2012), or trout instead of salmon (e.g. rainbow trout instead of Atlantic salmon; Cawthorn et al., 2012). The substitutions may encompass changes between species from different oceans (Table 1), the *Oncorhynchus* genus being native to the North Pacific and the *Salmo* genus native to the North Atlantic Ocean, although in the case of farmed species hatcheries can be located anywhere in temperate climates.

In this study we have analyzed samples of different salmonid products purchased from public marketplaces on the west coast of North America (Alaska in USA and Vancouver Island in Canada) and in Europe (Principate of Asturias, in the North of Spain, Bay of Biscay). We have performed the sampling in these places due to the high importance of salmonids products, being one of the most common fish type consumed and marketed in those regions (Hanner et al., 2011; Rasmussen et al., 2011; Larios, 1930). In addition, in these places the wild salmon species are very appreciated and with enormous cultural value, with several examples of ancestral traditions related with salmonids (Lynn et al., 2013; Valiente et al., 2011; Blanco et al., 2005). Due to such, we consider those regions as adequate for our study. The main aim of this work was to identify by DNA Barcoding methodology the species contained in salmon processed products, in order to assay the reliability of the labeling of such products in the two regions involved in the study. The expectations were that, in the case of existing mislabeling, any substitute species would be the nonnative and farmed species in each region, exchanging species from *Oncorhynchus* genus by *Salmo* genus and vice versa, which are the most common misidentifications detected in the other works (e.g.: Pardo et al., 2016 and Table 1).

2. Material and methods

2.1. Products analyzed

We obtained samples from local grocery stores in Alaska (USA), Vancouver Island (Canada), and Asturias (North of Spain), choosing one representative city in each target region, in which the salmon products market is high. All products purchased were processed products, being sold as: jerky, candy salmon, slices, loins, etc. (Table 2), and therefore being impossible to recognize the species by their morphological features.

In Alaska and Canada the analyzed products were salmon jerky and candy salmon, which are widely marketed and consumed in those regions. Furthermore, due to the high degree of processing of those samples, they are considered as a good niche for the species exchange,

being usually labeled only with the commercial common name, as was the case of the samples analyzed here (Table 2). A total of 31 products sold as salmon jerky were purchased in Anchorage, Alaska, USA from three local marketplaces in the summer of 2013 (Table 2). In Vancouver Island (Victoria, Canada), a total of 13 products were purchased, four salmon jerky and nine candy salmon pieces also from local grocery stores (4).

The samples from Asturias (Northwest Spain) (67) were purchased in six different grocery stores in Oviedo city (Asturias, Bay of Biscay), Spain. These products were more variable because in this region the consumption of processed salmonids products is similar between the different types. The majority of the samples (48) were heavily processed products (canned, smoked, salted, pâte) and the rest of the samples (19) were less processed products (fresh or frozen pieces).

In both regions the sampling was mainly focused on highly processed products since the mislabeling in those products may be higher (Muñoz-Colmenero et al., 2016; Filonzi et al., 2010; Rasmussen and Morrissey, 2008), and they are subject to more permissive laws than the products sold whole and fresh.

For all products and regions the expected species were determined from the information found on the labels and shown in Table 2.

2.2. DNA barcoding

DNA extraction was performed following the protocol developed by Estoup et al. (1996) using Chelex resin. DNA was extracted directly from a ~5 mm³ piece of fresh or frozen samples. All the other kind of products were previously cleaned with a 2:1:0.8 solution of distilled water, chloroform, and methanol in order to remove potential inhibitors of posterior PCR as oils, salts, etc., not present in frozen and fresh products. A fragment of the Cytochrome oxidase subunit I gene (COI) was amplified using the primers designed by Ward et al. (2005). The PCR conditions were: initial denaturation at 95 °C during 5 min followed by 35 cycles of 20 s at 95 °C, 20 s at 57 °C for annealing and 30 s at 72 °C for elongation. Finally, an extension step of 72 °C for 10 min. PCR was performed in 20 µL of total volume, and the composition was: 2.5 µM Mg²⁺, reaction buffer of Promega Taq polymerase 1 ×, 0.75 U Promega Taq enzyme (5U/µL), 2.5 mM of each dNTP, 1 µM primers, 2 µL of DNA, and bi-distilled water up to the total volume. PCR products were checked in 2% agarose gel stained with 3 µL ethidium bromide (0.5 µg/µL), purified with Illustra Exostar 1-Step (GE Healthcare Life Sciences), sequenced at the DNA Analysis Facility of the University of Oviedo with the BigDye Terminator Cycle Sequencing Kit v3.1 and analyzed on a 3130xl Genetic Analyzer (Applied Biosystems) Automated Sequencer.

The sequences were manually checked with the BioEdit v 7.0.9.0 Sequence Alignment Editor Software program (Hall, 1999). They were aligned using the Clustal W tool included in BioEdit (Thompson et al., 1994). To assign the species, the nucleotide BLAST tool (nBLAST) located in the GenBank public database from NCBI (<http://www.ncbi.nlm.nih.gov/>) was employed, with 99% of identity as cut-off. This cut-off is conservative enough to resolve the identification at the species level but keeps 1% for possible intraspecific variability. Those sequences in which mislabeling was found were also checked with the

Table 2

Salmonid products purchased. The total number of samples collected by place is showed in bold.

Place	Local Store	Product type	Processing type	Labeled name	N
Alaska (USA)	A1	Salmon Jerky	Jerky	Wild king salmon	18
	A2	Salmon Jerky	Jerky	Wild king salmon	7
	A3	Salmon Jerky	Jerky	Certified catchWild Alaskan salmon	6
					31
Vancouver Island (Canada)	V1	Salmon Jerky	Jerky	Chum salmon	4
	V2	Salmon candy	candy	Spring salmon	3
	V3	Salmon candy	candy	Spring salmon	3
	V4	Salmon candy	candy	Spring salmon	3
					13
Asturias (Spain)	S1	Paté	paté	Salmo salar	3
	S2, S3, S4	Smoked	smoked sheets	Salmo salar	33
	S4	Smoked	smoked sheets	Salmo trutta	3
	S5	Canned	canned pieces	Salmo salar	3
	S6	Salted	salted loins	<i>Oncorhynchus keta</i>	4
		Salted	salted loins	<i>Oncorhynchus gorbuscha</i>	2
	S2, S3	Fresh	slices	Norwegian salmon	9
		Fresh	without head and scrape	Rainbow trout	6
	S3	Fresh	without head and scrape	Brown trout	1
	S2	Frozen	frozen slices	Pacific <i>O. gorbuscha</i>	1
		Frozen	frozen fillets	Norwegian salmon	2
					67

Barcode of Life Database (BOLD; <http://www.boldsystems.org/>) to ensure our results.

Additional analysis to confirm species identification through neighbor-joining trees was conducted. Reference sequences of known species were downloaded from GenBank and aligned with the haplotypes found in our study. The best-fit evolution model for our dataset was determined by the program jModelTest 0.1.1 (Posada, 2008), using the Akaike information criterion. The tree was performed following the same criteria as Ardura et al. (2010), including the same nucleotide evolution model (Tamura-Nei) and replicates (2000), and employing MEGA version 6 (Tamura et al., 2013).

2.3. Statistical analyses

Mislabeleding was compared between samples using Chi-square contingency tests (χ^2) with the software included in Microsoft Excel (version 2010). Yates's correction (Yates, 1984) for small sample sizes was performed.

3. Results and discussion

All the products analyzed in this study except one sample from Alaska and one from Victoria (both salmon jerky) yielded DNA of enough quality for successful PCR amplification of the COI gene. This represents 98.2% success, and emphasizes the utility of DNA Barcoding for identification of fish species in processed seafood (Armani et al., 2015; Galal-Khallaif et al., 2014; Cawthorn et al., 2012; Wong and Hanner, 2008).

In total, after DNA analyses, we obtained 23 sequences of *Oncorhynchus tshawytscha* (from 33 expected), corresponding with six haplotypes (sequence variants), 23 sequences of *Oncorhynchus keta* (from 13 expected), corresponding with three haplotypes, 50 sequences of *Salmo salar* (from 50 expected), corresponding with six haplotypes, 3 sequences of *Oncorhynchus gorbuscha* (from 3 expected), corresponding with one haplotype, and 10 sequences of *Oncorhynchus mykiss* (from 4 expected), corresponding with two haplotypes. Lastly, no sequences were obtained for *Salmo trutta* (from 4 expected). All the haplotypes (18 in total) were submitted to the GenBank database, with the accession numbers KU756202-KU756219.

Our results revealed substitution in the salmon trade (Table 3), confirming previous studies (Table 1; Pardo et al., 2016). Barcoding unambiguously identified 14 mislabeled products that provided COI

sequences of high identity with GenBank and BOLD reference sequences of substitute species (Table 4). The phylogenetic analysis confirmed the results obtained from BLAST methodology, with the haplotypes of the mislabeled samples clustered with the substitute species (Fig. 1).

However, the proportion of mislabeled products was not identical in the two areas studied. It was 6% in Spanish samples and 23.8% in the samples obtained from northwest America (significant difference with $\chi^2 = 7.154$, d.f. = 1, $p < 0.01$). Indeed the type of products was different between locations, as Spanish samples included frozen and fresh products. When we removed these products (less processed) from the Spanish samples the percent mislabeling was still significantly lower than in America (7.3%; 3 mislabeled over 41 highly processed products, $\chi^2 = 4.904$, df = 1, $p < 0.05$).

The type of substitution was different in the two regions. In northwest America it was in all cases *Oncorhynchus keta* labeled and sold as *O. tshawytscha*, both products of wild catches, while the four Spanish cases were wild native brown trout *Salmo trutta* replaced by farmed rainbow trout *Oncorhynchus mykiss*. In Europe the mislabeling of the analyzed samples, as expected, corresponds to a change of wild species for farmed fish. Wild brown trout is a non-commercial species in the region (Spanish Law 6/2002 of June 18, available in Spanish at <https://www.boe.es/buscar/act.php?id=BOE-A-2002-15998>, accessed February 2016) and its catch is strictly restricted to sport fishing (angling), although it is unlikely that common consumers know this regulation. An enormous difference in price occurs between brown trout and substitute species in other countries where *Salmo trutta* can be commercialized; for example in Scotland loch trout (*Salmo trutta*) and rainbow trout (*O. mykiss*) are sold at 14.29 and 3.50 £/kg respectively in UK supermarkets (http://www.mysupermarket.co.uk/grocery-categories/Fresh_Salmon_And_Trout_in_Tesco.html, accessed February 2016). European consumers perceive farmed fish as more contaminated than wild fish (Pieniak et al., 2013), and it seems logical that any mislabeling try to please consumer preferences for wild fish.

In our North American samples the substitution was between species with wild catches, not wild-farmed. This may be explained by different prices of these two species. For example, in Alaska in 2013 the prices (average US\$/pound fish) were: King (*Oncorhynchus tshawytscha*) 6.72, versus Chum (*O. keta*) 1.50 (<http://www.alaskaseafood.org/industry/market/January2014SMB/salmon-price-report.html>, accessed February 2016). In a Washington State marketplace as much as 11% substitution of Pacific salmon for Atlantic salmon was found (Cline, 2012). The small sample size analyzed from the United States could explain the absence of this type of substitution in our study. However, the explanation could be

Table 3

Commercial products analyzed in North America and Spain. Cases of mislabeling are shaded in grey. The total number of products analyzed and the mislabeling found from each continent are marked in bold.

Region	Product	On the label	Type	Expected species	Real species from DNA	Real type	N	Mislabeling
Alaska	Salmon jerky	King salmon	Wild	<i>Oncorhynchus tshawytscha</i>	<i>Oncorhynchus keta</i>	Wild	1	
		King salmon	Wild	<i>Oncorhynchus tshawytscha</i>	<i>Oncorhynchus tshawytscha</i>	Wild	23	
		Alaska salmon	Wild	<i>Oncorhynchus keta</i>	<i>Oncorhynchus keta</i>	Wild	6	
Vancouver Island	Salmon candy	Spring salmon	Wild	<i>Oncorhynchus tshawytscha</i>	<i>Oncorhynchus keta</i>	Wild	9	
	Salmon jerky	Chum salmon	Wild	<i>Oncorhynchus keta</i>	<i>Oncorhynchus keta</i>	Wild	3	
					Total North America		42	23.8%
Spain	Paté	<i>Salmo salar</i>	Farmed	<i>Salmo salar</i>	<i>Salmo salar</i>	Farmed	3	
	Smoked	<i>Salmo salar</i>	Farmed	<i>Salmo salar</i>	<i>Salmo salar</i>	Farmed	33	
	Smoked	<i>Salmo trutta</i>	Wild	<i>Salmo trutta</i>	<i>Oncorhynchus mykiss</i>	Farmed	3	
	Canned	<i>Salmo salar</i>	Farmed	<i>Salmo salar</i>	<i>Salmo salar</i>	Farmed	3	
	Salted	<i>Oncorhynchus keta</i>	Wild	<i>Oncorhynchus keta</i>	<i>Oncorhynchus keta</i>	Wild	4	
	Salted	<i>Oncorhynchus gorboscha</i>	Wild	<i>Oncorhynchus gorboscha</i>	<i>Oncorhynchus gorboscha</i>	Wild	2	
	Fresh	Norwegian salmon	Farmed	<i>Salmo salar</i>	<i>Salmo salar</i>	Farmed	9	
	Fresh	Rainbow trout	Farmed	<i>Oncorhynchus mykiss</i>	<i>Oncorhynchus mykiss</i>	Farmed	6	
	Fresh	Brown trout	Wild	<i>Salmo trutta</i>	<i>Oncorhynchus mykiss</i>	Farmed	1	
	Frozen	Pacific <i>O. gorboscha</i>	Wild	<i>Oncorhynchus gorboscha</i>	<i>Oncorhynchus gorboscha</i>	Wild	1	
	Frozen	Norwegian salmon	Farmed	<i>Salmo salar</i>	<i>Salmo salar</i>	Farmed	2	
					Total Spain		67	5.97%

Table 4

DNA-based evidence of mislabeling found in this study. Haplotype, GenBank reference of the sequence found in our study. Percentage of coverage (%Cov) and identity (%Ide) with the closest match reference found in GenBank and BOLD by nBLAST methodology.

Region	Expected species	Real species from DNA	Haplotype	% Cov	% Ide	GenBank ref.	BOLD ref.
Alaska	<i>Oncorhynchus tshawytscha</i>	<i>Oncorhynchus keta</i>	KU756204	100	99	JX960914.1	AAA3872
Vancouver Island	<i>Oncorhynchus tshawytscha</i>	<i>Oncorhynchus keta</i>	KU756203	99	99	JX960914.1	AAA3872
			KU756204	100	99	JX960914.1	
			KU756205	100	99	JX960915.1	
Spain	<i>Salmo trutta</i>	<i>Oncorhynchus mykiss</i>	KU756207	99	100	JX960921.1	AAA1627

also economic. The price of farmed Atlantic salmon *Salmo salar* was higher than that of Chum salmon in Alaska (1.81 US\$/pound fresh fish; <http://www.agmrc.org/commodities-products/aquaculture/>, accessed February 2016). It would be economically advantageous to use Chum salmon, not Atlantic salmon, as substitute for king (spring) salmon. Although an exchange driven by economic reasons seems to make sense, we have to consider the option that the season is also an influence. Other studies such as Pardo et al., (2016) have mentioned that the substitution of

Oncorhynchus species by *Salmo* species is higher in winter, when the wild species are not available. In those months the sellers have only the cultivated species to do the exchanges.

Seafood supply is increasingly obtained farther from the consumption site (Watson et al., 2015). However, the substitute species found in this study are likely produced in situ, since *Oncorhynchus mykiss* is cultured in Europe, and the substitute species found in Northwest America have also local presence. To our surprise the results indicate

Acknowledgment

We are grateful to D. Serna for his help with laboratory tasks.

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