Occurrence of mislabelling in prepared fishery products in Southern Italy

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Abstract

Fish authentication is a major concern not only for the prevention of commercial fraud, but also for the assessment of safety risks deriving from the undeclared introduction of potentially dangerous toxic or allergenic substances or environmentally damaging fish where endangered species are involved. Moreover, food authentication might affect the diet of certain groups of consumers, such as followers of religious practices. Considering the authentication of fish products is one of the key issues in food safety, quality and sustainability, the aim of this work was to investigate the prevalence of mislabelling in sole (*Solea solea*), plaice (*Pleuronectes platessa*), Atlantic salmon (*Salmo salar*), and hake (*Merluccius merluccius*) fillets from markets and supermarkets located in Apulia (Southern Italy) using DNA barcoding. The results of the molecular investigations reveal that 42/98 (42.8%) fillet samples were not correctly labelled. In particular, 12/27 (44.4%) fillets of sole (*Solea solea*) were identified as belonging to *Solea senegalensis*. In addition, 13/28 (46.4%) plaice (*Pleuronectes platessa*) samples were identified as *Pangasius hypophtalmus*. All Atlantic salmon (*Salmo salar*) samples were correctly labelled. Post-sequencing data analysis revealed that 17/30 (56.6%) hake fillets (*Merluccius merluccius*) were not correctly labelled, of which 8/30 samples identified as *Merluccius hubbsi*, 5/30 samples as *Merluccius products* and 4/30 as *Merluccius capensis.* The study reveals a high occurrence of species mislabelling in the prepared fish fillet products, further evidence of the need for increased traceability and assessment of the authenticity of food products.

Introduction

The increasing demand for fishery products in general may lead to deliberate adulteration along the food chain, due to the substitution of high-quality species by lower quality counterparts. The authentication of prepared fish products is one of the key issues in food safety, quality and sustainability. Prepared fishery products, *i.e.* unprocessed fishery products that have undergone an operation affecting their anatomical wholeness are vulnerable to fraudulent labelling due to the economic profits arising from selling cheaper species as high-value ones (Di Pinto *et al.,* 2013). Moreover, different fish species may be similar in taste and texture, which makes it very difficult to identify the species correctly when the fish is delivered without its diagnostic body parts (*e.g.* skin, entrails, head and fins), or when it is turned into fillets or slices. Food authentication is a major concern not only for the prevention of commercial fraud, but also for the increased awareness among consumers regarding the composition of foods and the need to verify labelling statements (Bottero and Dalmasso, 2011; Armani *et al.,* 2015). In addition, fish substitution or mislabelling may be significant from a sanitary point of view because of potentially dangerous toxic or allergenic substances, or else environmentally damaging where endangered species are involved (Marko *et al.,* 2004; Ward *et al*., 2008; Wong and Hanner, 2008; Holmes *et al*., 2009). Moreover, food authentication might affect the diet of certain groups of consumers, such as followers of religious practices (Di Pinto *et al*., 2015, in press). Considering that seafood mislabelling has been reported throughout the world (Jacquet and Pauly, 2008; Cawthorn *et al*., 2011; Garcia-Vazquez *et al.,* 2011; Hanner *et al*., 2011; Cline, 2012; Miller *et al.,* 2012; Di Pinto *et al*., 2013, 2015) and that the authentication of food components is one of the key issues in food quality and safety, the aim of this study was to investigate the prevalence of mislabelling in sole (*Solea solea*), plaice (*Pleuronectes platessa*), Atlantic salmon *(Salmo salar)* and hake (*Merluccius merluccius*) fillets from markets and supermarkets located in Apulia (Southern Italy) using DNA barcoding (Hebert *et al*., 2003).

Materials and Methods

Sampling

A total of 98 samples of prepared fresh fillet fish products, including 27 sole (*Solea solea*), 28 European plaice (*Pleuronectes platessa*), 13 Atlantic salmon *(Salmo salar)* and 30 hake (*Merluccius merluccius*) from fish retail outlets fish retail premises, fish markets, supermarkets and hypermarkets located in Apulia (Southern Italy) were collected and stored at -20°C until processing. According to Council Regulation (EC) No 1379/2013 (European Comission, 2013) applicable from 01/01/2014, consumer labelling requirements (commercial designation, scientific name, production method and geographical area, whether previously frozen) were considered.

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DNA extraction and purification

Aliquots of each sample (10 mg) were subjected to DNA extraction and purification using the DNeasy Blood & Tissue Kit (QIAGEN, Hilden, Germany) as reported by Handy *et al*. (2011). Positive extraction controls were obtained from each specimen of authentic species. A negative extraction control (no added tissue) was included to verify the purity of the extraction reagents. The DNA concentration and purity were established by evaluating the ratio A260nm/A280nm using a Beckman DU-640B Spectrophotometer.

Oligonucleotide primers

The oligonucleotide primers, FISHCO1LBC: 5'-TCAACYAAT CAYAAAGATATYGGCAC-3' and FISHCO1HBC: 5'-ACTTCYGGGTGRCCR AARAATCA-3' reported by Handy *et al*. (2011) and synthesized by EUROFINS GENOMICS Srl (Milan, Italy), were used.

Polymerase chain reaction assay

The PCR reactions were performed in a final volume of 25 µL, using 12.5 µL of HotStarTaq Master Mix 2X (QIAGEN, Hilden, Germany), containing 2.5 units of HotStarTaq DNA Polymerase, 1.5 mM of $MgCl_2$ and 200 µL of each dNTP. Then, 1 µM of each oligonucleotide primer and 1 µL (40 ng/µL) of DNA were added. The amplification profile involved an initial denaturation step at 95°C for 15 min, followed by 30 cycles at 94°C for 30 s, 50°C for 40 s and 72°C for 60 s. The positive and negative controls for the extraction and PCR were included. The PCR reactions were processed in a Mastercycler Personal (Eppendorf, Milan, Italy). All reactions were performed in duplicate.

Detection of amplified products

PCR amplified products were analyzed by electrophoresis on 1.5% (w/v) agarose NA (Pharmacia, Uppsala, Sweden) gel in 1X TBE buffer containing 0.089 M Tris, 0.089 M boric acid, 0.002 M EDTA, pH 8.0 (USB, Cleveland, OH, USA), and stained with Green Gel Safe 10000X Nucleic Acid Stain (5 μL/100 mL) (Fisher Molecular Biology, USA). A Gene Ruler™ 100 bp DNA Ladder Plus (MBI Fermentas, Vilnius, Lithuania) was used as the molecular weight marker. Image acquisition was performed using UVITEC (Eppendorf).

Polymerase chain reaction cleanup

In order to produce an amplicon free of extra dNTPs and excess primers that might interfere with the sequencing reaction, the PCR products were purified with the QIAquick PCR Purification Kit (QIAGEN, Hilden, Germany).

Cycle sequencing reaction

Sequencing reactions using were performed as described by Handy *et al*. (2011) and carried out by EUROFINS GENOMICS Srl (Milan, Italy).

Sequence analysis

All amplified sequences were compared with sequences available in the Barcode of Life Data System (BOLD) and GenBank databases using Geneious Pro v5.4 (Drummond *et al.,* 2011). The bidirectional sequences with 98% HQ (98% high-quality bases) were compared with sequences from the BOLD and GenBank databases.

Results

The labels of only 37/98 fish fillet samples provided comprehensive information relating to the commercial designation, scientific name, geographical area, production method and whether they were previously frozen, according to the art. 35 of the Council Regulation (EC) n.1379/2013 (European Commission, 2013). The labelling of other samples was not compliant with European legislation. In particular, the scientific name was missing in 49/98 samples, the geographical area was omitted in 39/98, the commercial designation and the production method were reported in all samples.

The results of the molecular investigations reveal a high occurrence of incorrect species declaration in prepared fillet products (Table 1). Considering the Decree of the Italian Ministry of Agricultural, Food and Forestry Policies (MiPAAF) dated 31 January 2008, which reports the Italian name for fish species of commercial interest, the commercial and/or scientific name declared failed to match the species identified in 42/98 (42.8%) samples (Table 1). In particular, DNA of sufficient yield and quality was isolated and purified from all samples. The sequences obtained from the samples and compared against the BOLD and GenBank databases gave successful matches, varying from 98% to 100% pair wise sequence identity. Post-sequencing data analysis revealed that 42/98 (42.8%) fillet samples were not correctly labelled (Table 1). In particular, 12/27 (44.4%) fillets of sole (*Solea solea*) were identified as belonging to *Solea senegalensis*. In addition, 13/28 (46.4%) plaice (*Pleuronectes platessa*) samples were identified as *Pangasius hypophtalmus*. All Atlantic salmon (*Salmo salar*) samples were correctly labelled. Post-sequencing data analysis revealed that 17/30 (56.6%) hake fillets (*Merluccius merluccius*) were not correctly labelled, of which 8/30 samples identified as *Merluccius hubbsi,* 5/30 samples as *Merluccius products* and 4/30 as *Merluccius capensis.*

Discussion

In order to ensure high levels of safety, quality and transparency in seafood products, European Union food law implements the principle of quality management and process-oriented controls throughout the food chain – from the fishing vessel or aquaculture farm to the consumer's table. Although seafood labelling has to include the commercial designation, scientific name, geographical area, production method and state whether the product has been previously frozen, the commercial fish species available on the market cannot always be easily identified in processed and prepared fishery products, especially when morphological features have been removed.

Therefore, the current importance of the fish trade requires technological developments in food production, handling, processing and distribution by a global network of operators in order to guarantee the authenticity and the origin of fish and seafood products (Gil, 2007; Rasmussen and Morrissey, 2008; Di Pinto *et al*., 2013).

The results of this study reveal a high occurrence of incorrect species declaration in prepared fish fillet products, further evidence of the need for increased traceability and assessment of the authenticity of food products. In fact, cases of fraudulent mislabelling of lesser-valued species are becoming more common as commercial quotas on certain high-value species become more restrictive in the world (Barbuto *et al.,* 2010; Miller and Mariani, 2010; Cawthorn *et al*., 2011).

Fishery products substitution of valuable species of lower value is common practice because it is easy with a immediate economic reward. Generally, the species used in substitution have different and lower nutritional value compared with those declared as showed in this study. In addition, the substitution may be favored by the depletion in some areas of highly appreciated species, the high variety of fish species, the difficult differential diagnosis and the overall lack of taxonomical expertise. Moreover, fish identification may be insufficient if there are overlapping features between taxa, as it frequently occurs in many fish species (Di Pinto *et al*., 2015).

This study highlights the need for the sustainable management of aquatic resources, in particular, showed widespread use of species of lower commercial value and from highly polluted waters of African countries such as *Pangasius hypophthalmus* and *Merluccius capensis* respectively (Filonzi *et al*., 2010). Given the increase in consumption and production of convenience ready-to-cook seafood, precautionary measures are necessary. Traceability is an essential component of any risk management strategy, and a key requirement for post-marketing surveillance. The fishing industry requires a full traceability system, a crucial step in promoting greater seafood safety, quality and sustainability (Di Pinto *et al*., 2015).

Conclusions

Given the increasing demand for transparency in the food industry, the enforcement of proper labelling have provided a driving force for the development of suitable analytical methodologies for species identification. Indeed, the seafood industry currently lacks a simple, standardized, widespread method for tracing seafood products purchased along the supply chain. Specifically, DNA traceability could offer a more precise form of traceability for fish and byproducts, as provided by Council Regulation (EC) No 1224/2009, art. 13 (European Commission, 2009). A tracing system that combines genetic analysis with conventional methods of traceability may give food companies and consumers the information they need to make sustainable seafood choices. A great effort should therefore be made to create a strong standardized monitoring program or strategy and to evoke consumer awareness on several aspects of accurate labelling information (Di Pinto *et al*., 2015).

Table 1. Sole, plaice, hake and Atlantic salmon fillets results.

Sample number	Common name°	Latin name°	Catch location	Similarity (%)	True common name°	True Latin name° (COI ID.)	Genbank A.N.	Mislabelling
1	Sole	n.a	n.a	99	Sole	*Solea* solea	EU513746.1	No
2	Sole	*Solea* solea	FAO 37	99	Sole	*Solea* solea	EU513746.1	No
3	Sole	n.a	n.a	100	Atlantic sole	*Solea senegalensis*	KF3691186.1	Yes
4	Sole	n.a	FAO 37	99	Sole	*Solea* solea	EU513746.1	No
5	Sole	n.a	n.a	99	Atlantic sole	*Solea senegalensis*	KF3691186.1	Yes
6	Sole	*Solea* solea	FAO 27	99	Atlantic sole	*Solea senegalensis*	KF3691186.1	Yes
7	Sole	n.a	FAO 27	99	Sole	*Solea* solea	EU513746.1	No
8	Sole	n.a	FAO 27	99	Atlantic sole	*Solea senegalensis*	KF3691186.1	Yes
9	Sole	n.a	FAO 37	99	Sole	*Solea* solea	EU513746.1	No
10	Sole	*Solea solea*	n.a	99	Sole	*Solea* solea	EU513746.1	No
11	Sole	n.a	n.a	100	Atlantic sole	*Solea senegalensis*	KF3691186.1	Yes
12	Sole	n.a	FAO 27	99	Atlantic sole	*Solea senegalensis*	KF3691186.1	Yes
13	Sole	*Solea* solea	n.a	99	Sole	*Solea* solea	EU513746.1	No
14	Sole	*Solea* solea	n.a	99	Sole	*Solea* solea	EU513746.1	No
15	Sole	n.a	n.a	99	Atlantic sole	*Solea senegalensis*	KF3691186.1	Yes
16	Sole	*Solea* solea	FAO 27	99	Sole	*Solea* solea	EU513746.1	No
17	Sole	n.a	n.a	99	Atlantic sole	*Solea senegalensis*	KF3691186.1	Yes
18	Sole	*Solea* solea	n.a	99	Sole	*Solea* solea	EU513746.1	No
19	Sole	n.a	n.a	99	Atlantic sole	*Solea senegalensis*	KF3691186.1	Yes
20	Sole	*Solea* solea	FAO 27	99	Sole	*Solea* solea	EU513746.1	No
21	Sole	*Solea* solea	FAO 27	99	Sole	*Solea* solea	EU513746.1	No
22	Sole	n.a	FAO 37	99	Sole	*Solea* solea	EU513746.1	No
23	Sole	n.a	n.a	100	Atlantic sole	*Solea senegalensis*	KF3691186.1	Yes
24	Sole	*Solea* solea	FAO 27	99	Atlantic sole	*Solea senegalensis*	KF3691186.1	Yes
25	Sole	*Solea* solea	FAO 37	99	Sole	*Solea* solea	EU513746.1	No
26	Sole	*Solea* solea	n.a	99	Sole	*Solea* solea	EU513746.1	No
27	Sole	n.a	FAO 27	99	Atlantic sole	*Solea senegalensis*	KF3691186.1	Yes
28	Plaice	n.a	FAO 27	100	Plaice	*Pleuronectes platessa*	EU513682.1	No
29	Plaice	n.a	FAO 27	99	Plaice	*Pleuronectes platessa*	EU513682.1	No
30	Plaice *Pleuronectes platessa*		FAO 27	99	Pangasius	*Pangasius hypophtalmus*	JF292402.1	Yes
31	Plaice	n.a	FAO 27	99	Pangasius	*Pangasius hypophtalmus*	JF292402.1	Yes
32	Plaice *Pleuronectes platessa*		FAO 27	99	Pangasius	*Pangasius hypophtalmus*	JF292402.1	Yes
33	Plaice *Pleuronectes platessa*		FAO 27	99	Plaice	*Pleuronectes platessa*	EU513682.1	No
34	Plaice *Pleuronectes platessa*		FAO 27	98	Plaice	*Pleuronectes platessa*	EU513682.1	No
35	Plaice	n.a	FAO 27	98	Pangasius	*Pangasius hypophtalmus*	JF292402.1	Yes
36	Plaice *Pleuronectes platessa*		FAO 27	99	Plaice	*Pleuronectes platessa*	EU513682.1	No
37	Plaice *Pleuronectes platessa*		FAO 27	100	Plaice	*Pleuronectes platessa*	EU513682.1	No
38	Plaice *Pleuronectes platessa*		n.a	99	Pangasius	*Pangasius hypophtalmus*	JF292402.1	Yes
39	Plaice *Pleuronectes platessa*		FAO 27	99	Plaice	*Pleuronectes platessa*	EU513682.1	No
40	Plaice *Pleuronectes platessa*		FAO 27	99	Plaice	*Pleuronectes platessa*	EU513682.1	No
41	Plaice *Pleuronectes platessa*		FAO 27	99	Plaice	*Pleuronectes platessa*	EU513682.1	No
42	Plaice	n.a	FAO 27	99	Pangasius	*Pangasius hypophtalmus*	JF292402.1	Yes
43	Plaice	n.a	FAO 27	99	Plaice	*Pleuronectes platessa*	EU513682.1	No
44	Plaice	n.a	FAO 27	99	Pangasius	*Pangasius hypophtalmus*	JF292402.1	Yes
45	Plaice *Pleuronectes platessa*		FAO 27	99	Pangasius	*Pangasius hypophtalmus*	JF292402.1	Yes
46	Plaice *Pleuronectes platessa*		FAO 27	99	Plaice	*Pleuronectes platessa*	EU513682.1	No
47	Plaice *Pleuronectes platessa*		FAO 71	99	Plaice	*Pleuronectes platessa*	EU513682.1	No
48	Plaice	n.a	FAO 27	99	Pangasius	*Pangasius hypophtalmus*	JF292402.1	Yes
49	Plaice *Pleuronectes platessa*		FAO 27	100	Plaice	*Pleuronectes platessa*	EU513682.1	No
50	Plaice	n.a	n.a	99	Pangasius	*Pangasius hypophtalmus*	JF292402.1	Yes

Continued on next page

Table 1. Continued from previous page.

Sample number	Common name°	Latin name°	Catch location	Similarity (%)	True common name°	True Latin name° (COI ID.)	Genbank A.N.	Mislabelling
51	Plaice	n.a	n.a	99	Pangasius	*Pangasius hypophtalmus*	JF292402.1	Yes
52	Plaice	n.a	FAO 27	99	Pangasius	*Pangasius hypophtalmus*	JF292402.1	Yes
53	Plaice	*Pleuronectes platessa*	FAO 27	99	Plaice	*Pleuronectes platessa*	EU513682.1	No
54	Plaice	n.a	FAO 27	99	Plaice	*Pleuronectes platessa*	EU513682.1	No
55	Plaice	*Pleuronectes platessa*	n.a	99	Pangasius	*Pangasius hypophtalmus*	JF292402.1	Yes
56	Atlantic salmon	*Salmo salar*	FAO 27	99	Atlantic salmon	*Salmo salar*	FJ399413.1	No
57	Atlantic salmon	n.a	n.a	99	Atlantic salmon	*Salmo salar*	FJ399413.1	No
58	Atlantic salmon	*Salmo salar*	n.a	99	Atlantic salmon	*Salmo salar*	FJ399413.1	No
59	Atlantic salmon	*Salmo salar*	FAO 27	99	Atlantic salmon	*Salmo salar*	FJ399413.1	No
60	Atlantic salmon	*Salmo salar*	FAO 27	99	Atlantic salmon	*Salmo salar*	FJ399413.1	No
61	Atlantic salmon	n.a	FAO 27	99	Atlantic salmon	*Salmo salar*	FJ399413.1	No
62	Atlantic salmon	*Salmo salar*	FAO 27	99	Atlantic salmon	*Salmo salar*	FJ399413.1	No
63	Atlantic salmon	*Salmo salar*	n.a	99	Atlantic salmon	*Salmo salar*	FJ399413.1	No
64	Atlantic salmon	n.a	FAO 27	100	Atlantic salmon	*Salmo salar*	FJ399413.1	No
65	Atlantic salmon	*Salmo salar*	FAO 27	99	Atlantic salmon	*Salmo salar*	FJ399413.1	No
66	Atlantic salmon	*Salmo salar*	FAO 27	99	Atlantic salmon	*Salmo salar*	FJ399413.1	No
67	Atlantic salmon	n.a	n.a	99	Atlantic salmon	*Salmo salar*	FJ399413.1	No
68	Atlantic salmon	*Salmo salar*	n.a	99	Atlantic salmon	*Salmo salar*	FJ399413.1	No
69	Hake	*Merluccius merluccius*	FAO 37	98	Hake	*Merluccius merluccius*	FJ460768.1	No
70	Hake	*Merluccius merluccius*	FAO 37	99	Hake	*Merluccius merluccius*	FJ460768.1	No
71	Hake	n.a	n.a	99	Atlantic hake	*Merluccius hubbsi*	EU074472.1	Yes
72	Hake	*Merluccius merluccius*	n.a	99	Atlantic hake	*Merluccius hubbsi*	EU074472.1	Yes
73	Hake	*Merluccius merluccius*	FAO 37	99	Hake	*Merluccius merluccius*	FJ460768.1	No
74	Hake	n.a	n.a	98	Pacific hake	Merluccius productus	FJ164843.1	Yes
75	Hake	*Merluccius merluccius*	n.a	99	Hake	*Merluccius merluccius*	FJ460768.1	No
76	Hake	*Merluccius merluccius*	FAO 37	100	Hake	*Merluccius merluccius*	FJ460768.1	No
77	Hake	*Merluccius merluccius*	FAO 37	99	Hake	*Merluccius merluccius*	FJ460768.1	No
78	Hake	n.a	FAO 37	99	Atlantic hake	*Merluccius hubbsi*	EU074472.1	Yes
79	Hake	n.a	n.a	98	Pacific hake	Merluccius productus	FJ164843.1	Yes
80	Hake	n.a	n.a	99	Pacific hake	Merluccius productus	FJ164843.1	Yes
81	Hake	*Merluccius merluccius*	FAO 37	100	Hake	*Merluccius merluccius*	FJ460768.1	No
82	Hake	n.a	n.a	99	South African hake	*Merluccius capensis*	JF493884.1	Yes
83	Hake	*Merluccius merluccius*	FAO 37	99	Hake	*Merluccius merluccius*	FJ460768.1	No
84	Hake	*Merluccius merluccius*	FAO 37	99	Hake	*Merluccius merluccius*	FJ460768.1	No
85	Hake	n.a	n.a	99	South African hake	*Merluccius capensis*	JF493884.1	Yes
86	Hake	n.a	FAO 37	99	Hake	*Merluccius merluccius*	FJ460768.1	No
87	Hake	n.a	n.a	99	Hake	*Merluccius merluccius*	FJ460768.1	No
88	Hake	*Merluccius merluccius*	FAO 37	99	Hake	*Merluccius merluccius*	FJ460768.1	No
89	Hake	n.a	n.a	99	Pacific hake	Merluccius productus	FJ164843.1	Yes
90	Hake	n.a	FAO 37	100	Atlantic hake	*Merluccius hubbsi*	EU074472.1	Yes
91	Hake	n.a	n.a	99	Atlantic hake	*Merluccius hubbsi*	EU074472.1	Yes
92	Hake	n.a	n.a	99	Atlantic hake	*Merluccius hubbsi*	EU074472.1	Yes
93	Hake	*Merluccius merluccius*	FAO 37	99	Hake	*Merluccius merluccius*	FJ460768.1	No
94	Hake	n.a	n.a	99	South African hake	*Merluccius capensis*	JF493884.1	Yes
95	Hake	n.a	n.a	99	South African hake	*Merluccius capensis*	JF493884.1	Yes
96	Hake	n.a	n.a	99	Atlantic hake	*Merluccius hubbsi*	EU074472.1	Yes
97	Hake	n.a	n.a	99	Atlantic hake	*Merluccius hubbsi*	EU074472.1	Yes
98	Hake	n.a	n.a	98	Pacific hake	Merluccius productus	FJ164843.1	Yes

n.a, not available. °Latin and common name according to MiPAAF (2008).

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