



Short communication

Packaged frozen fishery products: species identification, mislabeling occurrence and legislative implications



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ABSTRACT

Given the increase in the international trade of packaged frozen fishery products, this study used DNA barcoding to investigate the breaded hake and plaice species, sold in Italian markets. The results of this study generally matched the ingredient list on the food label. Only 6 of the 120 samples were non-compliant. Specifically, breaded merluccius samples match the species shown in the list of ingredients on the label. Of the “breaded plaice” samples, 4/14 contained *Lepidopsetta polyxystra* and 2/14 *Merluccius gayi*, thus failing to match the ingredient list on the label. Considering the European legislation indicates that the label must not mislead consumers, but international trade and the use of similar terms for different products makes it complicated when a product from one country is introduced into another in which the niche already exists, clear labeling is strongly recommended in order to ensure that consumers can make conscious choices.

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1. Introduction

The fraudulent misdescription – with various undeclared species in ready-to-cook fish products – and commonplace adulteration of fish products with an unindicated mixture of fish, is an increasing and ever more widespread problem (Di Pinto et al., 2013; Di Pinto et al., 2015; Garcia-Vazquez et al., 2011; Premanandh, Sabbagh, & Maruthamuthu, 2013). Additionally, deep-frozen fish fillets and easy-to-prepare or ready-to-eat products have become the leading product, gradually outstripping the traditional salt fish and dried salt fish production. Indeed recent trends show an increase in the demand for and consumption of frozen fish, fillets and sashimi (thinly-sliced ready-to-cook seafood) purchased at supermarkets, at fish stores, and through home delivery services, while the consumption of fresh fish is decreasing (FAO, 2012; ISMEA, 2008).

The worldwide trade and processing of seafood have however increased the potential for species substitution on the commercial market (Hellberg and Morrissey, 2011). The effects of species substitution include economic fraud, health hazards, and the illegal trade in protected species (Carvalho, Neto, Brasil, & Oliveira, 2011). European regulations on the traceability and labeling of

food products require the food chain to be truly traceable, so as to inform consumers via obligatory product labeling and in order to create a “safety net” based on the traceability of these products at all stages of production and distribution to the marketplace.

Given the increase in the international trade of packaged frozen fishery products, the authentication of fish products is one of the most important areas concerning fish quality and safety and given the importance of the trade in processed fishery products, this study used DNA barcoding to investigate the hake and plaice species, labeled as breaded sticks, fillets, cutlets and croquettes sold in Italian markets.

2. Materials and methods

2.1. Sampling

A total of 120 packaged frozen fishery products were analyzed. Specifically, 120 samples of breaded fish, including 30 hake cutlets, 16 hake croquettes, 60 hake sticks and 14 breaded plaice fillets were from various frozen processed food market, supermarket and hypermarket chains located in Apulia (SE Italy), collected between September and November 2014 and stored at –20 °C until processed. Pursuant to European Union framework Regulation EC No 1169/2011, consumer labeling presentation and advertising of daily food requirements were considered.

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2.2. 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 and Deeds (2011). Briefly, aliquots (10 mg) of each fish sample were added to 50 μ L ATL lysis buffer and 5.56 μ L of Proteinase K (20 mg/mL) and incubated at 56 °C for 2 h. After adding 55.6 μ L AL Buffer and 55.6 μ L ethanol, the resulting mixture was applied to the DNeasy Mini spin column. The DNA, adsorbed onto the QIAamp silica-gel membrane during subsequent centrifugation steps at 6000 g for 1 min, was washed using 140 μ L AW1 and 140 μ L AW2 washing buffers. Finally, the DNA was eluted with 50 μ L AE Elution Buffer (QIAGEN, Hilden, Germany). 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. DNA concentration and purity were established by evaluating the ratio A260 nm/A280 nm using a Beckman DU-640B Spectrophotometer.

2.3. Oligonucleotide primers

The oligonucleotide primers, FISHCO1LBC: 5'-TCAACYAATCAY AAAGATATYGGCAC-3' and FISHCO1HBC: 5'-ACTTCYGGGTGCCRA ARAATCA-3' reported by Handy and Deeds (2011) and synthesized by PRIMM Srl (Milan, Italy), were used.

2.4. PCR assay

The PCR reactions were performed in a final volume of 25 μ L, using 12.5 μ L of HotStarTaq Master Mix 2X (QIAGEN, Hilden, Germany), containing 2.5 units of HotStarTaq DNA Polymerase, 1.5 mM of MgCl₂ and 200 μ L of each dNTP. Then, 1 μ M of each oligonucleotide primer and 1 μ L 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.

2.5. 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 μ L/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).

2.6. PCR 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).

2.7. Cycle sequencing reaction

Sequencing reactions using forward and reverse COI primers were performed by PRIMM Srl (Milan, Italy).

2.8. 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.

3. Results

DNA of sufficient yield and quality was isolated and purified from all 120 samples. All of these extractions resulted in PCR products clearly visible as single bands of expected size on agarose gel. The positive and negative controls for the extraction and PCR gave expected results. Next, the sequences obtained from the samples, compared against the BOLD and GenBank databases, gave successful matches varying from 98% to 100% pairwise sequence identity. All 120 interpretable sequences obtained in this study revealed *p*-distance values in accordance with taxonomic position. The results of the molecular investigations, reported in Table 1, generally matched the ingredient list on the food label. Only 6 of the 120 were non-compliant. Specifically, post-sequencing data analysis revealed that the 106/120 breaded fish, sold as “Merluccius cutlets”, “Merluccius croquettes” or “Merluccius sticks” tested as *Merluccius gayi*, *M. productus*, *Merluccius hubbsi*, *Merluccius senegalensis*, *Merluccius capensis*, *Merluccius paradoxus* and *Theragra chalcogramma*, matching the species shown in the list of ingredients on the label. Of the 14 “breaded plaice” samples, 8 samples were found to contain *Pleuronectes platessa* in accordance with the ingredient list on the food label and with the presentation, which refers to a specific species of fish, while 4/14 contained *Lepidopsetta polyxystra* and 2/14 *M. gayi*, thus failing to match the ingredient list on the food label and the presentation.

4. Discussions

Frozen processed food is an increasingly important key driver in the European food industry. While the volumes of processed fish products sold in the EU have fluctuated since 2008, their values have been steadily increasing at an average rate of 5% each year. In 2011, volumes of processed products sold in the EU totalled 4.26 million tonnes, equivalent to a value of EUR 16.8 billion. With respect to 2010, 2011 volumes registered an increase of 310,000 tonnes and values increased by more than EUR 1 billion (EUMOFA, 2014). Considering the increase in demand for processed fishery products and the reduction in EU resources, the sector is heavily dependent on imports. Thus hake and plaice, among the most important group of processed products sold in the EU, comes principally from fishing areas contaminated by heavy metals (Filonzi, Chiesa, Vaghi, & Marzano, 2010).

Furthermore, European hake (*Merluccius merluccius*) and European plaice (*P. platessa*) are species of great economic importance which make up a significant percentage of fish consumption in Italy, while species from the other genera show specific variances in organoleptic features, leading to differences in consumer preference and commercial value. The overfishing of stocks of commercial fish is a severe and complex problem around the world, with many well-known species including European hake (*M. merluccius*) and European plaice in serious decline. In fact, European hake (*M. merluccius*) and European plaice were among the most important group of processed products sold in the EU. However, the landed volumes of hake in the EU showed a remarkable 12% decrease, from more than 109,500 tonnes to around 97,000 tonnes in 2011 (EUMOFA, 2014). This decline was registered mainly in Spain and, to a lesser extent, in Italy and France,

Table 1
PCR results of processed fishery products.

Sample number	Commercial designation	Scientific name	Typology	COI-ID	Similarity (%)	Mislabeling
1	Hake cutlets	<i>Merluccius gayi</i>	Breaded fish	<i>Merluccius gayi</i>	98	No
2	Hake cutlets	<i>Merluccius gayi</i>	Breaded fish	<i>Merluccius gayi</i>	98	No
3	Hake cutlets	<i>Merluccius hubbsi</i>	Breaded fish	<i>Merluccius hubbsi</i>	99	No
4	Hake cutlets	<i>Merluccius capensis</i>	Breaded fish	<i>Merluccius capensis</i>	98	No
5	Hake cutlets	<i>Merluccius capensis</i>	Breaded fish	<i>Merluccius capensis</i>	99	No
6	Hake cutlets	<i>Merluccius gayi</i>	Breaded fish	<i>Merluccius gayi</i>	99	No
7	Hake cutlets	<i>Merluccius hubbsi</i>	Breaded fish	<i>Merluccius hubbsi</i>	99	No
8	Hake cutlets	<i>Merluccius hubbsi</i>	Breaded fish	<i>Merluccius hubbsi</i>	99	No
9	Hake cutlets	<i>Theragra chalcogramma</i>	Breaded fish	<i>Theragra chalcogramma</i>	98	No
10	Hake cutlets	<i>Theragra chalcogramma</i>	Breaded fish	<i>Theragra chalcogramma</i>	98	No
11	Hake cutlets	<i>Merluccius gayi</i>	Breaded fish	<i>Merluccius gayi</i>	98	No
12	Hake cutlets	<i>Merluccius gayi</i>	Breaded fish	<i>Merluccius gayi</i>	98	No
13	Hake cutlets	<i>Merluccius gayi</i>	Breaded fish	<i>Merluccius gayi</i>	99	No
14	Hake cutlets	<i>Merluccius productus</i>	Breaded fish	<i>Merluccius productus</i>	100	No
15	Hake cutlets	<i>Merluccius productus</i>	Breaded fish	<i>Merluccius productus</i>	98	No
16	Hake cutlets	<i>Merluccius productus</i>	Breaded fish	<i>Merluccius productus</i>	98	No
17	Hake cutlets	<i>Merluccius senegalensis</i>	Breaded fish	<i>Merluccius senegalensis</i>	98	No
18	Hake cutlets	<i>Merluccius paradoxus</i>	Breaded fish	<i>Merluccius paradoxus</i>	100	No
19	Hake cutlets	<i>Theragra chalcogramma</i>	Breaded fish	<i>Theragra chalcogramma</i>	100	No
20	Hake cutlets	<i>Merluccius senegalensis</i>	Breaded fish	<i>Merluccius senegalensis</i>	100	No
21	Hake cutlets	<i>Merluccius senegalensis</i>	Breaded fish	<i>Merluccius senegalensis</i>	98	No
22	Hake cutlets	<i>Merluccius paradoxus</i>	Breaded fish	<i>Merluccius paradoxus</i>	99	No
23	Hake cutlets	<i>Theragra chalcogramma</i>	Breaded fish	<i>Theragra chalcogramma</i>	99	No
24	Hake cutlets	<i>Merluccius senegalensis</i>	Breaded fish	<i>Merluccius senegalensis</i>	99	No
25	Hake cutlets	<i>Merluccius paradoxus</i>	Breaded fish	<i>Merluccius paradoxus</i>	99	No
26	Hake cutlets	<i>Merluccius gayi</i>	Breaded fish	<i>Merluccius gayi</i>	98	No
27	Hake cutlets	<i>Theragra chalcogramma</i>	Breaded fish	<i>Theragra chalcogramma</i>	99	No
28	Hake cutlets	<i>Merluccius capensis</i>	Breaded fish	<i>Merluccius capensis</i>	98	No
29	Hake cutlets	<i>Theragra chalcogramma</i>	Breaded fish	<i>Theragra chalcogramma</i>	99	No
30	Hake cutlets	<i>Merluccius capensis</i>	Breaded fish	<i>Merluccius capensis</i>	99	No
31	Hake croquettes	<i>Theragra chalcogramma</i>	Breaded fish	<i>Theragra chalcogramma</i>	98	No
32	Hake croquettes	<i>Merluccius gayi</i>	Breaded fish	<i>Merluccius gayi</i>	100	No
33	Hake croquettes	<i>Merluccius gayi</i>	Breaded fish	<i>Merluccius gayi</i>	98	No
34	Hake croquettes	<i>Merluccius capensis</i>	Breaded fish	<i>Merluccius capensis</i>	98	No
35	Hake croquettes	<i>Merluccius capensis</i>	Breaded fish	<i>Merluccius capensis</i>	99	No
36	Hake croquettes	<i>Merluccius capensis</i>	Breaded fish	<i>Merluccius capensis</i>	99	No
37	Hake croquettes	<i>Merluccius gayi</i>	Breaded fish	<i>Merluccius gayi</i>	98	No
38	Hake croquettes	<i>Merluccius hubbsi</i>	Breaded fish	<i>Merluccius hubbsi</i>	98	No
39	Hake croquettes	<i>Merluccius hubbsi</i>	Breaded fish	<i>Merluccius hubbsi</i>	98	No
40	Hake croquettes	<i>Merluccius paradoxus</i>	Breaded fish	<i>Merluccius paradoxus</i>	99	No
41	Hake croquettes	<i>Merluccius senegalensis</i>	Breaded fish	<i>Merluccius senegalensis</i>	99	No
42	Hake croquettes	<i>Merluccius hubbsi</i>	Breaded fish	<i>Merluccius hubbsi</i>	100	No
43	Hake croquettes	<i>Merluccius gayi</i>	Breaded fish	<i>Merluccius gayi</i>	100	No
44	Hake croquettes	<i>Merluccius gayi</i>	Breaded fish	<i>Merluccius gayi</i>	98	No
45	Hake croquettes	<i>Merluccius gayi</i>	Breaded fish	<i>Merluccius gayi</i>	98	No
46	Hake croquettes	<i>Merluccius hubbsi</i>	Breaded fish	<i>Merluccius hubbsi</i>	99	No
47	Hake stiks	<i>Merluccius senegalensis</i>	Breaded fish	<i>Merluccius senegalensis</i>	98	No
48	Hake stiks	<i>Merluccius hubbsi</i>	Breaded fish	<i>Merluccius hubbsi</i>	99	No
49	Hake stiks	<i>Merluccius productus</i>	Breaded fish	<i>Merluccius productus</i>	99	No
50	Hake stiks	<i>Merluccius paradoxus</i>	Breaded fish	<i>Merluccius paradoxus</i>	99	No
51	Hake stiks	<i>Merluccius productus</i>	Breaded fish	<i>Merluccius productus</i>	99	No
52	Hake stiks	<i>Merluccius senegalensis</i>	Breaded fish	<i>Merluccius senegalensis</i>	99	No
53	Hake stiks	<i>Merluccius hubbsi</i>	Breaded fish	<i>Merluccius hubbsi</i>	98	No
54	Hake stiks	<i>Merluccius paradoxus</i>	Breaded fish	<i>Merluccius paradoxus</i>	98	No
55	Hake stiks	<i>Merluccius hubbsi</i>	Breaded fish	<i>Merluccius hubbsi</i>	98	No
56	Hake stiks	<i>Merluccius gayi</i>	Breaded fish	<i>Merluccius gayi</i>	98	No
57	Hake stiks	<i>Merluccius paradoxus</i>	Breaded fish	<i>Merluccius paradoxus</i>	99	No
58	Hake stiks	<i>Merluccius paradoxus</i>	Breaded fish	<i>Merluccius paradoxus</i>	100	No
59	Hake stiks	<i>Merluccius hubbsi</i>	Breaded fish	<i>Merluccius hubbsi</i>	98	No
60	Hake stiks	<i>Theragra chalcogramma</i>	Breaded fish	<i>Theragra chalcogramma</i>	98	No
61	Hake stiks	<i>Merluccius hubbsi</i>	Breaded fish	<i>Merluccius hubbsi</i>	99	No
62	Hake stiks	<i>Merluccius paradoxus</i>	Breaded fish	<i>Merluccius paradoxus</i>	98	No
63	Hake stiks	<i>Merluccius hubbsi</i>	Breaded fish	<i>Merluccius hubbsi</i>	99	No
64	Hake stiks	<i>Theragra chalcogramma</i>	Breaded fish	<i>Theragra chalcogramma</i>	99	No
65	Hake stiks	<i>Merluccius hubbsi</i>	Breaded fish	<i>Merluccius hubbsi</i>	99	No
66	Hake stiks	<i>Merluccius hubbsi</i>	Breaded fish	<i>Merluccius hubbsi</i>	98	No
67	Hake stiks	<i>Theragra chalcogramma</i>	Breaded fish	<i>Theragra chalcogramma</i>	98	No
68	Hake stiks	<i>Merluccius hubbsi</i>	Breaded fish	<i>Merluccius hubbsi</i>	100	No
69	Hake stiks	<i>Merluccius senegalensis</i>	Breaded fish	<i>Merluccius senegalensis</i>	100	No
70	Hake stiks	<i>Merluccius capensis</i>	Breaded fish	<i>Merluccius capensis</i>	100	No
71	Hake stiks	<i>Merluccius hubbsi</i>	Breaded fish	<i>Merluccius hubbsi</i>	98	No
72	Hake stiks	<i>Theragra chalcogramma</i>	Breaded fish	<i>Theragra chalcogramma</i>	99	No
73	Hake stiks	<i>Theragra chalcogramma</i>	Breaded fish	<i>Theragra chalcogramma</i>	98	No
74	Hake stiks	<i>Merluccius hubbsi</i>	Breaded fish	<i>Merluccius hubbsi</i>	99	No

(continued on next page)

Table 1 (continued)

Sample number	Commercial designation	Scientific name	Typology	COI-ID	Similarity (%)	Mislabeling
75	Hake stiks	<i>Merluccius hubbsi</i>	Breaded fish	<i>Merluccius hubbsi</i>	99	No
76	Hake stiks	<i>Merluccius senegalensis</i>	Breaded fish	<i>Merluccius senegalensis</i>	98	No
77	Hake stiks	<i>Merluccius hubbsi</i>	Breaded fish	<i>Merluccius hubbsi</i>	98	No
78	Hake stiks	<i>Merluccius hubbsi</i>	Breaded fish	<i>Merluccius hubbsi</i>	98	No
79	Hake stiks	<i>Merluccius hubbsi</i>	Breaded fish	<i>Merluccius hubbsi</i>	98	No
80	Hake stiks	<i>Merluccius capensis</i>	Breaded fish	<i>Merluccius capensis</i>	99	No
81	Hake stiks	<i>Merluccius hubbsi</i>	Breaded fish	<i>Merluccius hubbsi</i>	100	No
82	Hake stiks	<i>Theragra chalcogramma</i>	Breaded fish	<i>Theragra chalcogramma</i>	99	No
83	Hake stiks	<i>Merluccius hubbsi</i>	Breaded fish	<i>Merluccius hubbsi</i>	98	No
84	Hake stiks	<i>Merluccius hubbsi</i>	Breaded fish	<i>Merluccius hubbsi</i>	98	No
85	Hake stiks	<i>Theragra chalcogramma</i>	Breaded fish	<i>Theragra chalcogramma</i>	99	No
86	Hake stiks	<i>Theragra chalcogramma</i>	Breaded fish	<i>Theragra chalcogramma</i>	99	No
87	Hake stiks	<i>Merluccius hubbsi</i>	Breaded fish	<i>Merluccius hubbsi</i>	99	No
88	Hake stiks	<i>Theragra chalcogramma</i>	Breaded fish	<i>Theragra chalcogramma</i>	100	No
89	Hake stiks	<i>Merluccius gayi</i>	Breaded fish	<i>Merluccius gayi</i>	98	No
90	Hake stiks	<i>Merluccius hubbsi</i>	Breaded fish	<i>Merluccius hubbsi</i>	99	No
91	Hake stiks	<i>Merluccius paradoxus</i>	Breaded fish	<i>Merluccius paradoxus</i>	99	No
92	Hake stiks	<i>Merluccius paradoxus</i>	Breaded fish	<i>Merluccius paradoxus</i>	98	No
93	Hake stiks	<i>Merluccius hubbsi</i>	Breaded fish	<i>Merluccius hubbsi</i>	98	No
94	Hake stiks	<i>Merluccius hubbsi</i>	Breaded fish	<i>Merluccius hubbsi</i>	100	No
95	Hake stiks	<i>Theragra chalcogramma</i>	Breaded fish	<i>Theragra chalcogramma</i>	99	No
96	Hake stiks	<i>Merluccius hubbsi</i>	Breaded fish	<i>Merluccius hubbsi</i>	98	No
97	Hake stiks	<i>Theragra chalcogramma</i>	Breaded fish	<i>Theragra chalcogramma</i>	98	No
98	Hake stiks	<i>Merluccius gayi</i>	Breaded fish	<i>Merluccius gayi</i>	98	No
99	Hake stiks	<i>Merluccius hubbsi</i>	Breaded fish	<i>Merluccius hubbsi</i>	99	No
100	Hake stiks	<i>Merluccius gayi</i>	Breaded fish	<i>Merluccius gayi</i>	99	No
101	Hake stiks	<i>Merluccius gayi</i>	Breaded fish	<i>Merluccius gayi</i>	100	No
102	Hake stiks	<i>Merluccius senegalensis</i>	Breaded fish	<i>Merluccius senegalensis</i>	100	No
103	Hake stiks	<i>Merluccius hubbsi</i>	Breaded fish	<i>Merluccius hubbsi</i>	100	No
104	Hake stiks	<i>Merluccius senegalensis</i>	Breaded fish	<i>Merluccius senegalensis</i>	99	No
105	Hake stiks	<i>Merluccius gayi</i>	Breaded fish	<i>Merluccius gayi</i>	99	No
106	Hake stiks	<i>Merluccius hubbsi</i>	Breaded fish	<i>Merluccius hubbsi</i>	98	No
107	Breaded plaice	<i>Pleuronectes platessa</i>	Breaded fish	<i>Pleuronectes platessa</i>	98	No
108	Breaded plaice	<i>Pleuronectes platessa</i>	Breaded fish	<i>Merluccius gayi</i>	100	Yes
109	Breaded plaice	<i>Pleuronectes platessa</i>	Breaded fish	<i>Pleuronectes platessa</i>	100	No
110	Breaded plaice	<i>Pleuronectes platessa</i>	Breaded fish	<i>Pleuronectes platessa</i>	98	No
111	Breaded plaice	<i>Pleuronectes platessa</i>	Breaded fish	<i>Lepidopsetta polyxystra</i>	99	Yes
112	Breaded plaice	<i>Pleuronectes platessa</i>	Breaded fish	<i>Lepidopsetta polyxystra</i>	99	Yes
113	Breaded plaice	<i>Pleuronectes platessa</i>	Breaded fish	<i>Merluccius gayi</i>	99	Yes
114	Breaded plaice	<i>Pleuronectes platessa</i>	Breaded fish	<i>Pleuronectes platessa</i>	100	No
115	Breaded plaice	<i>Pleuronectes platessa</i>	Breaded fish	<i>Pleuronectes platessa</i>	98	No
116	Breaded plaice	<i>Pleuronectes platessa</i>	Breaded fish	<i>Lepidopsetta polyxystra</i>	99	Yes
117	Breaded plaice	<i>Pleuronectes platessa</i>	Breaded fish	<i>Pleuronectes platessa</i>	99	No
118	Breaded plaice	<i>Pleuronectes platessa</i>	Breaded fish	<i>Pleuronectes platessa</i>	99	No
119	Breaded plaice	<i>Pleuronectes platessa</i>	Breaded fish	<i>Pleuronectes platessa</i>	98	No
120	Breaded plaice	<i>Pleuronectes platessa</i>	Breaded fish	<i>Lepidopsetta polyxystra</i>	100	Yes

with each of the three markets reflecting the supply reduction in different ways.

Although different hake and plaice species may be similar in taste and texture and it is very difficult to identify the species correctly when the product is delivered, especially once filleting and other forms of de-characterization have eliminated key morpho-anatomical characters, this study underlines a generally achievement of an effective tracing system in frozen processed fishery products industry and an adequate application of HACCP plan especially in hake products industry. Indeed, the correct application of HACCP represents an important approach to managing food safety and food authentication in food production, by performing adequate selection of suppliers' procedures, which are crucial in the food sector and specifically in the frozen fishery products industry. Therefore, the adoption and implementation of more stringent quality control and traceability procedures by suppliers – including regular DNA testing – are urgently needed in order to determine the accuracy of the labels used for processed fishery products.

Also the results of this study underline the need to strengthen European legislation related to labeling in the processed fishery products field. Indeed, in the seafood field, EC Regulations

introduced two new requirements: (i) the provision of specific consumer information at the point of retail sale to the final consumer, whether sold loose or prepacked; (ii) the maintenance of a traceability trail of required information throughout all stages of the distribution and wholesale chain. The labeling of fish at each stage of marketing is governed by Council Regulation (EC) No. 1379/2013 on the common organization of the markets in fishery and aquaculture products and Commission Regulation (EC) No. 1420/2013 laying down detailed rules for the application of Council Regulation (EC) No. 1379/2013 as regards informing consumers about fishery and aquaculture products. Member States are required to establish commercial designations for species that shall be used when applying Article 35 of 1379/2013. In Italy, the list of agreed commercial designations for use is contained in the Decree of the Italian Ministry of Agricultural, Food and Forestry Policies (MiPAAF) dated 31 January 2008. The list indicates the scientific (Latin) name for each species, its name in the official language or languages of the Member State and, where applicable, any other name or names accepted or permitted locally or regionally.

However, the above Regulations apply exclusively to live fish, fresh, chilled or frozen fish, fillets of fish, smoked fish, salted and dried fishery products as well as live or raw shellfish, such as

oysters or mussels, raw or cooked whole prawns and shrimps. By contrast, processed products such as frozen and breaded fillets or ready-to-eat dishes are not covered by the above Regulations, which do not apply to fish to which other ingredients have been added such that the fish becomes an intrinsic part of the end-product, coated/battered/breaded fish products, recipe dishes/fish ready meals, or to fish that has been further processed, preserved, treated or cooked. In fact, Reg. EC 1169/11, *Annex VII*, Part B establishes that “All species of fish where the fish constitutes an ingredient of another food and provided that the name and presentation of such food does not refer to a specific species of fish” may be designated by the name of that category rather than the species name, and so may be labeled simply as “fish”. Therefore, the generic name, “fish”, can be used in the ingredients list instead of a more specific name to describe any species of fish, but only if the label does not refer to a specific species of fish.

Consequently, this study highlights that fish species are incorrectly given to consumers. In particular, the results indicate non-compliance with labeling products. Indeed, the results of the molecular investigations resulted in all 106/120 hake-based products matching the ingredient list on the food label, but not with the common name of the food printed in boldface type on the main display panel of the package in Italian. The name and presentation refer to a specific species of fish, *M. merluccius*, denominated as “merluzzo” by the Decree of the Italian Ministry of Agricultural, Food and Forestry Policies (MiPAAF), was never detected.

In Italy, however, the Ministry Circular of 4 March 2011 (Assoittica Italia, 2013), on labeling of packaged frozen fishery products, allows producers to name the product using the generic term “merluzzo” even when other species in the same family are used, provided these are shown on the list of ingredients. Thus, pre-packaged foodstuffs must comply with the rules on labeling, presentation and advertising of foodstuffs, ensuring product authenticity and correct consumer information. Also the legislation should consider the sanitary aspects, species-specific fish allergens (Rona et al., 2007; Sharp and Lopata, 2014; Taylor, Kabourek, & Hefle, 2004; Woo and Bahna, 2011) linked to use of the generic name, “fish”, which can be used in the ingredients list instead of a more specific name to describe any species of fish. Furthermore, factors such as traceability, quality, and sustainability criteria have become an integral part of the sale contract requirements, thus leading to changes in the selection of raw material by the processing industry.

In general, the legislation of all countries indicates that the label must not mislead consumers, but international trade and the use of similar terms for different products makes it complicated when a product from one country is introduced into another in which the niche already exists. Different species may have the same common name in different countries. On the other hand, sometimes the same species has different names in the same language at different locations in the same country. Considering the international trade of processed seafood, there is a need to harmonise at European Union (EU) level to enable European consumers to make informed choices and to remove obstacles to the free circulation of foodstuffs and unequal conditions of competition. However, further information is also required in compound fish products. In addition, there is a need to extend mandatory origin labeling for other foods such as fish used as an ingredient in breaded fish products. The indication of the country of origin clearly identified on the label in order to stimulate consumption of local products and avoid particular fishing areas (e.g. over fears of contamination by heavy metals).

5. Conclusions

Considering the increasing demand for transparency with regard to the geographical origin of the product, its wild or farmed origin, health and sustainability issues, clear labeling is strongly recommended in order to ensure that consumers can make conscious choices. Enforcing European legislation guidelines would be associated with the development and application of reliable labeling implementation plans and appropriate traceability systems in order to guarantee an efficient food safety system (Marín, Fujimoto, & Arai, 2014). Also, a continuous monitoring scheme along with improved detection methodologies and stringent sanctions on defaulters may help to minimize authentication problems in future. Therefore, the food control authorities may upgrade their systems to identify food sources and monitor quality to ensure that proper processing has taken place and labeling information reflects actual contents. The need for regular and safe supply is a clear opportunity for fish farming to further develop. Innovation and promotion will be key elements of the competitiveness of the fishing industry, in the future. It will also be important to look in detail at the industry's specific concerns and to ensure a better match between fish supply and the real needs of the fish market.

References

- Assoittica Italia. 2013. Anno XXV, 117, 2–4.
- Carvalho, D. C., Neto, D. A. P., Brasil, B. S. A. F., & Oliveira, D. A. A. (2011). DNA barcoding unveils a high rate of mislabeling in a commercial freshwater catfish from Brazil. *Mitochondrial DNA*, 1, 97–105.
- Di Pinto, A., Di Pinto, P., Terio, V., Bozzo, G., Bonerba, E., Ceci, E., et al. (2013). DNA barcoding for detecting market substitution in salted cod fillets and battered cod chunks. *Food Chemistry*, 141, 1757–1762.
- Di Pinto, A., Marchetti, P., Mottola, A., Bozzo, G., Bonerba, E., Ceci, E., et al. (2015). Species identification in fish fillet products using DNA barcoding. *Fisheries Research*, 170, 9–13.
- Drummond, A. J., Ashton, B., Buxton, S., Cheung, M., Cooper, A., Duran, C., et al. (2011). Geneious v5.4 2011. <<http://www.geneious.com/>>.
- EUMOFA (European Market Observatory for Fisheries and Aquaculture Products). 2014. <<http://www.ec.europa.eu/fisheries/market-observatory>>.
- FAO (Food and Agriculture Organization of the United Nations) (2012). World review of fisheries and aquaculture. Fisheries and Aquaculture Department Food and Agriculture Organization of the United Nations, Rome, 3–307. <<http://www.fao.org/home/en/>>.
- Filonzi, L., Chiesa, S., Vaghi, M., & Marzano, F. N. (2010). Molecular barcoding reveals mislabelling of commercial fish products in Italy. *Food Research International*, 43, 1383–1388.
- García-Vázquez, E., Perez, J., Martinez, J. L., Pardiñas, A. F., Lopez, B., Karaïskou, N., et al. (2011). High level of mislabeling in Spanish and Greek hake markets suggests the fraudulent introduction of African species. *Journal of Agricultural and Food Chemistry*, 59, 475–480.
- Handy, S. M., & Deeds, J. R. (2011). A single-laboratory validated method for the generation of DNA barcodes for the identification of fish for regulatory compliance. *Journal of AOAC International*, 94, 201–210.
- Hellberg, R. S., & Morrissey, M. T. (2011). Advances in DNA-Based techniques for the detection of seafood species substitution on the commercial market. *Journal of Laboratory Automation*, 16, 308–321.
- ISMEA (Institute of services for the agricultural and food market) (2008). The fisheries sector in Italy. Check-up 2008 p 1–5. <<http://www.ismea.it/>>.
- Marín, A., Fujimoto, T., & Arai, K. (2014). The variable 5' end of the 16S rRNA gene as a novel barcoding tool for scallops (Bivalvia, Pectinidae). *Fisheries Science*, 81, 73–81.
- Premanandh, J., Sabbagh, A., & Maruthamuthu, M. (2013). Misdescription of packaged foods: a case study from the United Arab Emirates. *Food Additives & Contaminants: Part A*, 30, 2022–2026.
- Rona, R. J., Keil, T., Summer, C., Gislason, D., Zuidmeer, L., & Sodergren, E. (2007). The prevalence of food allergy: a meta-analysis. *Journal of Allergy Clinical Immunology*, 120, 638–646.
- Sharp, M. F., & Lopata, A. L. (2014). Fish Allergy. In Review. *Clinical Reviews in Allergy & Immunology*, 46, 258–271.
- Taylor, S. L., Kabourek, J. L., & Hefle, S. L. (2004). Fish allergy: Fish and products thereof. *Journal of Food Science*, 69, 175–180.
- Woo, C. K., & Bahna, S. L. (2011). Not all shellfish “allergy” is allergy! *Clinical and Translational Allergy*, 1(1), 7.