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Detection of fish species substitution frauds in Italy: A targeted National Monitoring Plan

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ABSTRACT

Fighting food frauds is a ceaseless challenge because of the constant evolution of fraudulent practices and for the consequences both on consumers' and on globalized trade. In Italy fish is a vulnerable commodity for frauds thanks to the high national production, importation and consumption and it is important to monitor the entire food chain in order to detect and prevent fraudulent actions, such as species substitutions, which is considered the most common fraud in seafood.

Aim of this study was to realise a targeted Monitoring Plan to estimate the prevalence of fish species substitutions in Italy. As a first step, Italian fish supply chain, from production to selling, was analysed, in order to identify products and chain points at risk, by reviewing literature and by involving, in two focus groups, food inspectors and representatives of the large scale food distribution system. Then a monitoring plan was designed by sampling three fish species considered at major risk for their economic value and/or large consumption, i.e. tuna, grouper and flat fishes, at different selling points (wholesale markets, retail markets, fish shops and supermarkets). From February to March 2017, 242 samples from fresh, frozen or transformed whole fishes or fillets were sampled in 13 Italian cities (5 in Northern Italy, 2 in Central Italy, 3 in Southern Italy and 3 in the main Islands). Samples were analysed by "FINS" (Forensically Informative Nucleotide Sequencing), using two markers: cytochrome oxidase subunit I gene (*COI*) as first option and then mitochondrial cytochrome B gene (*cytb*), if necessary to identify uncertain or unassigned samples. Species substitutions were uncovered in 8.7% of analysed samples, principally related to grouper (prevalence of 14.71%). Supermarkets resulted the selling points with a major number of frauds (prevalence of 12.79%). Substituted species were taxonomically related to those declared on the label and no species harmful for consumers were detected. Results obtained can give to National Authorities a detailed frame of trends in fish substitution frauds in Italy, providing also relevant information to put into effect control measures.

1. Introduction

Frauds are a matter of concern in many food products and fish, as reported in the European Parliament Resolution of 14 January 2014 on "the food crisis, fraud in the food chain and the control thereof", is identified at a major risk for fraudulent actions in Europe (EU, 2014). European and National legislation dealing with traceability and labelling of food products has contributed to prevent and control frauds; these legislative instruments at European level are represented by general laws, such as Regulation 178/2002 and Regulation 1169/2011, and specific laws, such as Regulation 1379/2013, that provides important information about labelling of seafood products, with particular attention to origin and name of species (EU, 2002; EU, 2011; EU, 2013). At Italian level, these issues have been also regulated by a specific criminal code article (IT, 2017) and by administrative laws, such as Legislative Decree 109/1992 on food labelling - partially abrogated

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after the introduction of Regulation 1169/2011 and modified by Legislative Decree 231/2017 (IT, 2017) - and Legislative Decree 206/2005 (Consumer Code) (IT, 1992; IT, 2005, p. 235). Besides adequate legislative tools, it is also necessary to implement an efficient and effective control system in order to check law compliance, to suddenly detect frauds and to protect consumers' health. In fact, food frauds cannot be considered only as an economic issue because they can represent also a threat for human health (e.g. substitution of safe fish species with toxic ones). In this context, it is important to correctly analyse the food chain, identify risk points, organise monitoring plans to understand the current situation and review control strategies according to results obtained, in a perspective of a continuous improvement.

Mislabelling, and in particular species substitution, i.e. selling a species different from that declared on the label, are the most frequent frauds in seafood. In 2015 the European Commission organised a control plan to assess the prevalence on the market of white mislabelled fish with regard to its declared species (EU, 2015).

The results of this European Plan can be an important starting point to focus on this phenomenon also with initiatives at National level, designing targeted Monitoring Plans and Control strategies based on the specific risks of each country.

Therefore, the objective of this study was to organise and perform a targeted Italian Monitoring Plan to estimate the prevalence of fish substitution frauds in Italy. The Plan was based on the identification of critical points in the fish supply chain, through the analysis of national production data and consumption tendencies. Species and places at major risk were assessed exploiting the information derived from this analysis.

2. Materials and methods

2.1. Focus groups on production process and control activities

Between May and June 2016, two focus groups were organised with the participation of veterinarians working on food safety and authenticity in the "Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta" (IZSPLVA), a National public veterinary laboratory, of representatives of a company operating in the organised large scale food distribution system and of two inspectors operating in seafood products supply chain control. During the meetings, the principal phases of seafood supply chain (production, transformation, distribution) were analysed in order to detect the most common substitution frauds, that both stakeholders and controllers usually have to face, and risk points related.

2.2. Literature review of Italian production, import and consumption data of fish products

Different reports written by National and European Statistical Institutes from 2011 to 2015 and other publications (EU, 2006–2016; EUMOFA, 2015; CREA, 2015; ISMEA, 2015; ISMEA, 2013; ISMEA, 2011) were evaluated to obtain information about:

- Italian fishery and aquaculture production (global production value, caught fish volumes and principal caught species, farmed fish volumes and principal farmed species, principal transformed products);
- import (volumes of imported products, most imported products and countries of origin);
- average selling prices of most consumed products
- most consumed species;

The objective of this information gathering was to identify products that are more likely to be substituted because of at least one of the following characteristics:

- low availability;
- high price;
- high consumption.

2.3. Revision of previous monitoring and control plans

In the frame of the European Control Plan of 2015 (EU, 2015) in Italy, 251 samples of fresh and processed products of white fishes (hake/cod, rhombus, sole, plaice, halibut, sea bream, sea bass, grouper, perch, sturgeon), obtained from canteens, restaurants, supermarkets, marketplaces, processing plants, border inspection posts, were analysed.

The results of this Plan were reviewed in order to obtain further information regarding species, products and supply chain points at major risk, to be accounted for in the sampling design of the National Monitoring Plan of the present study.

2.4. Sampling design of the plan

Considering the results of the above described phases, three fish categories (grouper, tuna, flat fish) and four sampling points (wholesale markets, retail markets, fish shops and supermarkets) were chosen to be included in the Plan, for the following reasons:

grouper was chosen because of its high commercial value; some species are caught in the Mediterranean Sea while others are imported from foreign countries, such as Argentina, Senegal and Vietnam. Moreover, it is normally sold as fillets or prepared products, losing its morphologic characteristics and making difficult to distinguish it from less valuable species. Additionally, the European Plan of 2015 revealed that grouper was the most frequently substituted species;

tuna was one of the principal imported seafood products in Italy in 2015. *Thunnus thynnus* is the most valuable species but its fish stocks have diminished in the last years, therefore every European country involved in fishing has got its own catch rate; *Thunnus albacares* is less valuable and it is the species normally used for canned tuna fish. Therefore, it is possible that *Thunnus thynnus* would be substituted with *Thunnus albacares* to sell the product with a higher price, by using additives to give to *Thunnus albacares* the red colour of *Thunnus thynnus* (many RASFF alerts from 2006 to 2017 refer to additives or CO treatment in tuna) (EU, 2006–2016); another possibility is that *Thunnus albacares* would be substituted by *Thunnus thynnus* illegally caught, or that canned tuna is produced using species not allowed by Regulation 1536/92 (EU, 1992);

flat fish, sole and plaice above all, are normally sold as fillets, difficult to discriminate. Moreover, a study conducted by sampling fresh and frozen European plaice and common sole fillets in the Southern Italy revealed that 35% of plaice samples and 41% of sole samples were mislabelled (Pappalardo & Ferrito, 2015); while considering the results of the European Plan of 2015, 2 non-compliances were detected in Italy in 34 samples of flat fishes (5.9%) (EU, 2015).

Regarding sampling points, from focus group sessions and literature review, the importance of distribution phase emerged as a major risk point for substitution frauds, because in some cases it is the last part of the supply chain before consumption, and in other cases (for example referring to wholesale markets that supply products to supermarkets) it is the unique external phase that is difficult to control by supermarkets' quality systems. For this reason, different distribution points were chosen to be included in the sampling plan.

Therefore, the current Plan was designed based only on literature review for tuna and on both literature review and results of previous Plans for grouper and flat fish.

Sample size was assessed as follows:

- Thirty-nine fresh or frozen fillets of grouper. The number was established in order to detect a minimum national percentage of substitution of 7.5% with a confidence level of 95%; the minimum

percentage reported was calculated to be lower than that derived from the previous study (EU, 2015);
- Ninety-nine fresh or frozen slices of tuna fish or canned tuna; the number was established in order to detect a minimum national percentage of substitution of 3% with a confidence level of 95%; this low minimum percentage was arbitrary established as no previous National studies were available;
- Ninety-nine fresh or frozen fillets of flat fishes (preferably rhombus, sole, plaice); the number was established in order to detect a minimum national percentage of substitution of 3% with a confidence level of 95%; the minimum percentage reported was calculated to be lower than that derived from previous studies (EU, 2015).

2.5. Sampling process

Sampling was performed by official controllers (Anti Adulteration Nucleus of National Crime Agencies) in 13 different Italian cities representing the North, the Centre and the South of the country, including the two main islands (Sicily and Sardinia): Ancona, Bari, Bologna, Cagliari, Catania, Genova, Livorno, Milano, Napoli, Palermo, Pescara, Torino and Treviso. In every sampled place, aliquots of 10 gr or more of fish were taken, stored at −20 °C and sent to the Genetics Laboratory of the IZSPLVA, in Turin, to be analysed.

From February to March 2017, in different selling places, a total of 242 samples were collected: 34 samples were declared as grouper, 101 declared as tuna fish and 107 declared as flat fish, Depending on products availability in different cities and selling points, a sample size slightly bigger than that previously established was collected for tuna and flat fishes while a sample number slightly smaller was obtained for grouper.

Sampling division between species, cities and sampling points is summarised in Table 1.

2.6. DNA extraction and PCR

DNA was extracted from 25 mg of tissue using a commercial kit based on silica purification (ReliaPrep™ gDNA Tissue Miniprep System, Promega). All the samples were analysed amplifying a portion of *COI* gene in a PCR reaction containing Platinum™ Quantitative PCR SuperMix-UDG 1X (Invitrogen) and 0.3 μM of each primer taken from Ward, Zemlak, Innes, Last, and Hebert (2005), but assembling the two forward and the two reverse primers into a set of degenerated primers. Fifty-one samples out of those analysed did not amplified, so subsequently they were tested with *cytb* gene (Verma & Singh, 2003). Mainly, these samples belonged to *Thunnus albacares*, *Psetta maxima*, and *Solea vulgaris*.

2.7. DNA sequencing

Amplicons were sequenced using Sanger method on both strands and *consensus* sequences were compared to those reported in public databases BLAST (http://blast.ncbi.nlm.nih.org) and, only for those obtained from *COI* genes, in BOLD Identification System (www.boldsystems.org) too. The species was assigned on the basis of a similarity with sequences reported in public databases ≥ 98%. According to the FINS (Forensically Informative Nucleotide Sequencing) method, when similarity values were the same for more than one species, a phylogenetic tree was built and a bootstrap test was associated, in order to evaluate statistical significance (Terol, Mascarell, Fernandez-Pedrosa, & Perez-Alonso, 2002). FINS is a widely used method for species identification, based on the comparison of the nucleotide sequence of a genetic marker, for an unknown sample, with reference sequences. To this, a phylogenetic analysis is added to value the distance matrix and to construct a phylogenetic tree, if necessary (Santaclara, Pérez-Martín, & Sotelo, 2014).

2.8. Elaboration of results

Results obtained from molecular analysis were transferred into an Excel file, to calculate the total percentage of non-compliances, and the percentage of non-compliances by species and by sampling point, with their confidence intervals.

3. Results

3.1. Focus groups on production process and control activities

During the focus group sessions, both stakeholders and controllers agreed that species substitution mainly occurs during distribution phases, mostly involving expensive and transformed fish, such as grouper and tuna fish.

3.2. Literature review of Italian production, import and consumption data of fish products

Concerning production, literature review revealed that canned tuna is one of the main transformed products in Italy, but also one of the most imported product, primarily coming from Spain. Regarding consumption, in 2015 Italy was the 4th European country with the highest per capita expense in fish foodstuffs (EUMOFA, 2016) Moreover, considering consumers' preferences, especially for people living in cities far from sea, preference are for fish products in supermarkets than in other sale points (EU, 2006–2016; EUMOFA, 2015; CREA, 2015; ISMEA, 2015; ISMEA, 2013; ISMEA, 2011).

Table 1
Number of samples of the Monitoring National Plan divided for species, point and city of sampling.

City	Total number of sampled products	Groupers	Tuna fishes	Flat fishes	Wholesale markets	Retail markets	Fish shops	Supermarkets
Milano	16	3	7	6	6	3	3	4
Palermo	8	1	4	3	0	3	0	5
Torino	19	3	8	8	11	0	0	8
Treviso	18	1	9	8	7	4	3	4
Ancona	23	2	8	13	7	6	7	3
Livorno	21	2	12	7	7	0	1	13
Bologna	21	4	8	9	0	1	10	10
Cagliari	15	1	9	5	5	5	2	3
Pescara	19	3	8	8	4	0	0	15
Catania	21	7	8	6	10	0	4	7
Napoli	18	2	8	8	9	1	4	4
Bari	18	0	9	9	0	2	8	8
Genova	25	5	3	17	9	0	1	15
Totale	242	34	101	107	75	25	43	99

3.3. Revision of previous monitoring and control plans

Considering Italian results of the European Control Plan for fish substitution organised in 2015, 6 samples (2.4%) were non-compliant with species declaration, with 4 of these declared as grouper, and substituted with taxonomically unrelated species, and 2 declared as flat fishes, but substituted with similar species. Four non-compliant samples derived from border inspection posts and two from retail (EU, 2015).

3.4. Current National Monitoring Plan

Concerning results of the Plan performed in the present study, after molecular analysis 12 samples were excluded because they could not be amplified: 11 out of 24 analysed canned tuna didn't originate a PCR amplification, probably due to the heat treatment to which these products are subjected, which can cause DNA fragmentation. One flat fish (*Arnoglossus laterna*) could also not be amplified: before this project the laboratory didn't have experience about its PCR amplification being these the first samples analysed belonging to this species. Although claimed to be universal, it is possible that the primers used in this work are not able to amplify this flat fish.

Therefore 230 samples were sequenced and among these, 20 non-compliant results were detected (8.69% - C.I. 95%:5.4–13.1%) Considering examined species, 5 non-compliances were detected in groupers (14.7% of analysed grouper samples - C.I. 95%:5–31%), 7 in tuna fish (6.93% of analysed tuna fish samples - C.I. 95%:2.8–13.8%), 8 in flat fishes (7.55% of analysed flat fishes - C.I. 95%:3.3–14.3%; Table 3).

None of these frauds involved toxic or dangerous species and detected species were always taxonomically related to those declared on the label.

List of non-compliances is summarised in Table 2.

Supermarkets were the places with the highest risk to detect frauds, with 11 non-compliances out of 99 samples analysed (11.11%, C.I. 95%: 5.7%-19%) while Catania is the city with the highest number of non-compliances (5/21 samples).

Detailed results of the Plan are summarised in Table 3.

4. Discussion

In this study an integrated approach was applied to examine fish supply chain, identify risk points for species substitutions and set up a targeted National Monitoring Plan for fish species substitution.

According to the obtained results, grouper resulted the species with the highest rate of substitutions. This finding confirms results of previous studies conducted in the Southern part of Italy: in Salerno, species identification performed on 18 transformed fish samples revealed 3 substitutions, one of them involving grouper and represented by replacement of *Epinephelus marginatus* with *Reinhardtius hippoglossoides* (Cutarelli et al., 2014).

Table 2
Non-compliances revealed during the Monitoring Plan.

Declared species	Detected species	Number of frauds
Epinephelus aereolatus	*Epinephelus sp*	1
Epinephelus costae	*Mycteroperca rubra*	2
Acanthistius brasilianus	*Macrourus carinatus*	1
Epinephelus diacanthus	*Epinephelus undulosus*	1
Lepidorhombus boscii	*Lepidorhombus whiffagonis*	1
Raja radiata	*Raja clavata*	1
Paralichtys isosceles	*Paralichtys patagonicus*	1
Solea vulgaris	*Solea senegalensis*	2
Arnoglassus laterna	*Lepidorhombus boscii*	1
Dicologoglossa cuneata	*Pegusa cadenati*	1
Solea senegalensis	*Solea vulgaris*	1
Thunnus albacares	*Thunnus obesus*	6
Thunnus albacares	*Thunnus thynnus*	1

Frauds were detected in flat fish too, although in percentages lower than those uncovered in other studies: in particular, a work carried out in Apulia (Southern Italy) analysed 27 soles and 28 European plaices, resulting in 12 (44%) *Solea senegalensis* labelled as *Solea solea* and 13 (46%) *Pangasius hypophtalmus* labelled as *Pleuronectes platessa* (Tantillo et al., 2015). In the present study instead, in the same region (Bari district) 2/9 (22,2%) frauds were detected, both involving flat fishes, although the percentage of substitutions was lower than the previous one and other species than sole and plaice were involved (*Lepidorhombus boscii* labelled as *Arnoglassus laterna* and *Pegusa Cadenati* as *Dicologoglossa cuneata*), suggesting that also other less valuable species, probably less controlled, can be substituted.

In another study conducted in Calabria and Sicily regions (Southern Italy), 5/15 (33,3%) soles and 10/25 (40%) plaices sampled in supermarkets resulted mislabelled (Pappalardo and Ferrito, 2015), while in part of the same area (Catania district) in the present work an higher percentage (50%) of sole samples collected in supermarkets and fish shops resulted adulterated while no plaices were mislabelled. In both studies sole was substituted with similar less valuable fishes (respectively *Arnoglossus laterna* and *Solea senegalensis*).

Absence of frauds in plaice occurred in the present study can be explained by a continuous improving of fish stocks in Northern Sea and therefore an increasing availability of fish also in winter, when fishing is stopped for plaice reproductive period.

Regarding tuna, results confirmed the trend revealed in other countries such as Spain (the main exporter of tuna in Italy), where, in a study conducted collecting for a year (2014–2015) tuna samples from wholesale markets, fishmongers and supermarkets, 37% of substituted samples were detected, involving substitution of *Thunnus albacares* with *Tunnus obesus, Thunnus thynnus* with *Thunnus albacares* and, during *Thunnus Thynnus* fishing season (from May to July), reverse substitutions of *Thunnus albacares* with *Thunnus thynnus* (Gordoa et al., 2017). In the present study the majority of adulterations referred to the first type (6.7%) due to deception of fishing stocks of *Thunnus albacares* and a minor part (1%) to reverse substitution, probably due to illegal fishing.

Moreover, in none of the examined categories harmful species were detected. It is possible that this risk could be higher in ethnic shops and at border inspection points, that could be considered in future monitoring and control activity.

Considering results according to sampling points, supermarkets appeared to be the distribution points with the highest number of frauds, probably because many transformed products are sold there, and they are more difficult to recognise than fresh fish sold in the markets. In fact, also in previous studies high percentages of substitution frauds were detected in samples obtained from supermarkets: in 2008, 32% of 69 samples of different fish species, collected in department stores and fishmongers in 4 regions in the Northern and Central Italy, resulted mislabelled (Filonzi, Chiesa, Vaghi, & Nonnis Marzano, 2010).

Finally, referring to results of the entire current National Plan in comparison with Italian results obtained from European Plan of 2015, the total number of substitutions was higher in the current plan while in both Plans grouper was detected as the species with the highest percentage of substitutions. These findings could be explained by differences in sampling points and species due to different purposes. In fact, the European Plan could be considered as an explorative study involving all the phases of fish supply chain from production to trade and considering a large number of species suitable to be sampled all over Europe. The present study instead, based on the results of the European Plan but also on other sources, designed a more specific Plan targeted on Italian reality, by choosing species and sampling points considered at major risk in that context.

Table 3
Distribution of non-compliances detected in the National Monitoring Plan with percentages and confidence intervals.

City	Total NC	Grouper NC	Tuna fish NC	Flat fish NC	Wholesale market NC	Retail market NC	Fish shop NC	Supermarket NC
Milano	1	0	1	0	0	0	0	1
Palermo	1	0	0	1	0	1	0	0
Torino	1	0	1	0	1	0	0	0
Treviso	3	1	2	0	1	0	0	2
Ancona	0	0	0	0	0	0	0	0
Livorno	2	1	0	1	0	0	0	2
Bologna	2	1	0	1	0	0	0	2
Cagliari	0	0	0	0	0	0	0	0
Pescara	1	1	0	0	0	0	0	1
Catania	5	1	2	2	2	0	1	2
Napoli	1	0	1	0	1	0	0	0
Bari	2	0	0	2	0	0	1	1
Genova	1	0	0	1	1	0	0	0
Total	20	5	7	8	6	1	2	11
Totanalysis	230	34	101	107	75	25	43	99
%	8.69	14.7	6.93	7.55	8.00	4.00	4.65	11.11
CI 95%	5.4%-13.1%	5%-31%	2.8%-13.8%	3.3%-14.3%	3%-16.6%	0.1%-20%	0.6%-15.8%	5.7%-19%

5. Conclusions

Fish substitutions are among the most important frauds in seafood products.

Therefore, it is important to obtain information on risk points and prevalence of this phenomenon, in order to perform a correct strategy to detect illicit practices. Our study is one of the few Plans on fish substitutions that can provide a picture of this phenomenon not at local but at national level. The preliminary assessment of productive processes and consumption tendencies, along with the review of previous monitoring activities, appeared to be an effective way to better target Monitoring Plans and to obtain results suitable for risk management and fraud control.

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