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DNA barcoding revealing mislabeling of seafood in European mass caterings

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ABSTRACT

Seafood is amongst the most internationally traded food commodities worldwide and it is one of the food groups most likely to be subject to fraud. A number of studies have been conducted where samples from retail-, restaurant- and food service outlets have been tested for species substitution. These studies have mostly focused on specific species, particular types of outlets or confined to some geographical location. The study presented in this paper is the first large-scale attempt to study the rate of fish mislabeling in mass caterer (HoReCa) sector across Europe. A total of 283 samples were collected in 180 mass caterer outlets in 23 European countries. DNA barcoding revealed that 26% of the samples were mislabeled and that 31% of the outlets sold mislabeled seafood. The highest mislabeling rate was observed in Spain, Iceland, Finland and Germany, where close to 50% of the outlets sampled offered mislabeled seafood. Conversely, there was no mislabeling detected in Sweden, Switzerland and Slovakia. The species with the highest mislabeling rates were dusky grouper, butterfish, pike perch, sole, bluefin tuna and yellowfin tuna. In the case of other important fish species in Europe such as hake, cod, haddock and swordfish, mislabeling rates ranged between 14 and 33%. The results of the study show that the majority of the mislabelings are with cheaper fish, such as the presence of Pangasius commonly substituting other species, being labeled as more expensive ones, suggesting economic motivation for mislabelling.

1. Introduction

Global food trade has expanded enormously in the last decades, and fish and fishery products are amongst the most internationally traded food commodities worldwide. To be able to meet this demand, seafood production has been growing over the last decades, with special emphasis in aquaculture production, accounting for 45% of global seafood production in 2015 (FAO, 2015). According to the Food and Agriculture Organization (FAO), the world seafood overall production was close to 170.4 million tonnes in 2015: 93.7 million tonnes of captures (inland and marine) and 76.6 million tonnes of total aquaculture (FAO, 2016). Moreover, in 2012 it was estimated that 50% of the world's fisheries were at maximum exploitation levels, and nearly 25% were overexploited (FAO, 2016). Besides, IUU (Illegal, Unreported and Unregulated fishing) can lead to overexploitation of fish stocks, hampering their recovery, therefore becoming an important global threat.

Mislabeling and inaccurate identification of species in fish landings or modifying the capture area also contributes to underreported exploitation of stocks and the consequent reduction of fishery resources. This lack of control plays an important role in the threatening of fisheries sustainability despite international efforts, and can even imply the eventual extinction of the most vulnerable overexploited species (Agnew et al., 2009).

There is also an inherent public health implication, since substitute species may be potentially harmful, such as some oilfish species (Cabrero, Hernández, Tango, Hillera, & Marcos, 2015) and puffer fish species *Lagocephalus spp.*, known to contain the neurotoxin tetrodotoxin (TTX) (Armani et al., 2015), or certain species more allergenic than others (Triantafyllidis et al., 2010).

European Union labeling law states that seafood products must be labeled with the complete scientific name of the species (i.e.

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genus and species, Latin binomial nomenclature) ("Regulation (EU) No 1379/2013 f11 December 2013 on the Common Organization of the arkets in fishery and aquaculture products, amending Council Regulations (EC)No 1184/2006 and (EC) No 1224/2009 and repealing Council Regulation (EC)No 104/2000. OJEU 2013; L354.,"). The commercial name of some species can encompass several species, varying across different countries, and even different regions. This can hinder consumer's choice since in some cases, species with different market prices can be marketed under the same commercial name. The lack of non-harmonized commercial names categorization across different jurisdictions fosters mislabeling. In this sense, the use of recognizable names both locally and internationally is desired to ensure sufficient traceability in the seafood production chain (Armani, Castigliego, & Guidi, 2012).

Reliable analytical methods are needed for species identification in order to detect mislabeling. In this sense, DNA forensic analysis methods can be applied when visual methods are inadequate for species identification. DNA barcoding by means of PCR and sequencing of specific mitochondrial DNA fragments (*12S rRNA*, *COI*, *CYTB* and *DLOOP*) is the most commonly used approach in species identification of seafood products (Griffiths et al., 2014).

Deliberate misdescription and replacement of high value species by lower value species for economic reasons is an economically motivated adulteration (EMA) and should be considered as fraud (Spink & Moyer, 2011). In a policy paper published in 2013 by the European Parliament, seafood was identified as the second most likely group of food to be subjecti to fraud, following olive oil (Committee on the Environment: Draft report on the food crisis, fraud in the food chain and the control thereof, 2013). This issue becomes especially important in a sector as vulnerable as mass-catering business. According to Reg. (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011, 'mass caterer' means any establishment (including a vehicle or a fixed or mobile stall), such as restaurants, canteens, schools, hospitals and catering enterprises in which, in the course of a business, food is prepared to be ready for consumption by the final consumer ("Regulation (EU) No 1169/2011 of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004. OJEU 2011; L304.,"). Since morphological characteristics such as fins, skin and heads, are lost when the fish is processed, and other characteristics such as colour might be unstable after freezing or cooking, the consumer might be unable to verify whether what they are eating corresponds with that stated on the menu. Although the legislation mentioned above is not specifically intended for restaurants and canteens, and scientific species denomination is not compulsory in restaurants, according to regulation EC No 1169/2011 ("Regulation (EU) No 1169/2011 of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004. OJEU 2011; L304.,"), the information provided should meet transparency requirements regarding ingredients employed. Consumers' awareness of environmental and nutritional aspects of the products they consume has raised notably. Consumers demand more information and transparency regarding the food they acquire, to be able to make informed choices (Logan, Alter, Haupt, Tomalty, & Palumbi, 2008).

In specific links of the seafood production chain, such as processing industries, it is highly unlikely that mislabeling should take place by accident, since, in general, workers deal constantly with the same species (Miller, Jesse, & Mariani, 2012). As a matter of fact, the vast majority of studies (90%) focused their sampling efforts at the retail end of the supply chain, mainly supermarkets and fishmonger while few studies (10%) used samples from hotels, restaurants and catering (Pardo, Jiménez, & Pérez-Villarreal, 2016) revealing high variable degrees of mislabeling (Bénard-Capelle et al., 2015; Cawthorn, Duncan, Kastern, Francis, & Hoffman, 2015; Christiansen, Fournier, Hellemans, & Volckaert, 2018; Kappel & Schröder, 2016; Khaksar et al., 2015; Vandamme et al., 2016). This fact could have an explanation if we consider what it means to take samples in hotels, bars, caterings and restaurants throughout a specific region or country. To overcome this constraint, citizen science is a relatively new approach that can be very useful to increase the sampling coverage. This approach was successfully applied in a fish survey on restaurants and supermarkets in Paris (France) (Bénard-Capelle et al., 2015) and it implemented laboratory practices of genetics for providing real samples of food products consumed by students at home (Borrell, Munoz-Colmenero, Dopico, Miralles, & Garcia-Vazquez, 2016). In this sense, to be able to collect as many samples as possible, and in the largest number of countries, we mobilized dozens of citizens/samplers in this collaborative work to assess the extent of seafood mislabeling in European restaurants. In this study, a large-scale samples collection has been performed in restaurant in 23 states across Europe for the first time. The objective of this study was to answer the following question "which is the percentage of restaurants that sold seafood different to the name indicated on the menu?". The mislabeling rate was assessed by comparing the molecular results to the commercially accepted names in the respective country.

2. Materials and methods

2.1. Sampling

Samples were collected from mass-catering establishments all over Europe from 2015 to 2016. A representative sampling plan was uniformly designed in 23 European countries: Portugal, Spain, Finland, Baltic States (Latvia, Lithuania and Estonia), France, Sweden, Italy, Belgium, Netherlands, Denmark, Greece, Cyprus, United Kingdom, Ireland, Germany, Slovenia, Czech Republic and Romania. Furthermore, Iceland was also included in the study because it is the country with the highest fish consumption in Europe (Table 1). The survey was also complemented with some samples from Switzerland and Norway. Samples were collected by more than 100 scientists as collectors through the personal connections of the authors and participants from EU FoodIntegrity project (https://secure.fera.defra.gov.uk/foodintegrity/index.cfm) and Labelfish following a detailed protocol. Participant collectors were required to include a small portion of the seafood in the provided tube which included RNA*later*™ stabilization solution (Invitrogen). Where possible, the seafood samples were taken from the central area of the portion to reduce the risk of contamination with sauces and heat damage to the DNA. Data such as commercial name detailed on the menu, date, price, establishment and address were collected. Price category was selected subjectively according to the standard of living of the country where the samples were taken: low, medium and high. Commercial name was recorded in the original language or in English when the menu was requested in this language by the collector. To avoid bias, the sampling was neither limited by the origin (wild vs farmed), nor by market preferences.