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Short communication

Amplification of 16S rDNA reveals important fish mislabeling in Madrid restaurants

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ABSTRACT

Food fraud encompasses economic fraud and can produce health problems for consumers, as well as conservation problems for the involved species. Nevertheless, few studies tested for mislabeling in restaurants. In this study, we tested for mislabeling of fish species in restaurants. We sampled 77 fish dishes from 53 different restaurants located in 9 different districts of Madrid, Spain. A short fragment of the 16S rDNA was employed for DNA amplification leading to species or genera identification. Results showed that 7 species or genera and almost 30% of the samples were mislabeled. Mislabeling was present in 37% of the sampled restaurants and in 71% of the sampled districts. Mislabeling was common and it was not correlated with a districts' economic status (i.e. with the official average square-meter price of apartments). The analyses also showed that some species were more prone to be mislabeled than others.

1. Introduction

Food fraud can have important consequences for consumers (e. g. Spink & Moyer, 2011). The most obvious consequences for consumers are of economic nature. Intentional adulteration of food usually provides the vendor with a financial advantage, what is known as 'Economic Motivated Adulteration (EMA) of food' (Everstine, Spink, & Kennedy, 2013). Another consequence is the inadvertent consumption of species, which can produce serious health problems (Triantafyllidis et al., 2010), and that may even lead to intoxication (e.g. with TTX; Giusti et al., 2018). Last but not least, food fraud can also produce important problems for species conservation (e.g. Ward, Holmes, & Last, 2008), since it includes protected species, endangered species and species with capture quota. In these cases, food fraud generally masks the illegal exploitation (Horreo, Machado-Schiaffino, & Garcia-Vazquez, 2017; Pramod, Nakamura, Pitcher, & Delagran, 2014). Food fraud must be avoided, and its detection and the knowledge about how, where, and when food fraud occurs, is the first step to control it.

Seafood is extremely important in the European Union (EU). For example in 2015, the EU seafood supply (domestic production and import) reached more than 14.5 million tons, and households spent 54.8 billion Euros on seafood from fisheries and aquacultures (EUMOFA, 2017). Food fraud usually occurs due to financial incentives (von der Heyden, Barendse, Seebregts, & Matthee, 2010), and it can be detected using cost-effective, DNA based analytical methods (Tinacci et al., 2018). Fish fraud can happen during catching, at the wholesaler, during processing (Muñoz-Colmenero, Blanco, Arias, Martinez, & Garcia-Vazquez, 2016), in end-user markets (Muñoz-Colmenero et al., 2015) or in restaurants. In restaurants, the percentage of mislabeling is suggested to be higher than in supermarkets and retailers (Bérnard-Capelle et al., 2015), but few studies exist so far and specific studies are required in order to confirm these suggestions (Pardo, Jiménez, & Pérez-Villarreal, 2016; Pardo et al., 2018). In order to detect fish fraud, the fish species indicated on the product label (bought in a shop/supermarket/supplier/restaurant) can be compared with the fish species revealed by genetic analyses (e.g. Muñoz-Colmenero et al., 2016). Fish can be mislabeled along the entire supply chain, i.e. when indicating where it was caught (in which geographic area, in the sea, or in a fish farm), during the acquisition of intermediate buyers, during processing, up to the place where the end-user buys it. This makes the origin of mislabeling difficult to track. For example, a restaurant may unintentionally purchase a wrongly labeled species, it may unintentionally use the wrong fish for preparing a meal, or it may intentionally change the species' name to increase his benefits (Kappel & Schröder, 2016), thereby defrauding the restaurant's guests. The detection of mislabeling is very important in order to alert and act against no-ethic and illegal

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behavior but unfortunately, as commented above, studies investigating mislabeling in restaurants are scarce.

Madrid is the capital of Spain (Europe) and Spain is the country with the highest household expenditure for fisheries and aquaculture in the European Union (EUMOFA, 2017). Three million inhabitants live in the center of the city and Madrid is Europe's 2nd biggest city. Madrid is one of the world's most touristic capitals, with more than 5.5 million tourists visiting Madrid per year (International, 2017). The hostelry is very important and the city center consists of 2862 restaurants and 3307 bars (Servilab, 2010) and to our knowledge, no studies assessed the prevalence of food fraud in this city except in grouper (Asensio, 2008). In this study, we tested for mislabeled food and measured the prevalence of mislabeling in fish by comparing whether species and genera indicated on a label coincided with the fish species unraveled by genetic methods. Anonymous clients took tissue samples from fish meals in several restaurants located in different districts/areas in the city of Madrid and species identification was done employing DNA amplification and BLAST analysis. Results will provide evidence for the existence or absence of fish mislabeling in restaurants of one of the biggest cities of Europe.

2. Materials and methods

2.1. Sampling

A total of 77 different fish samples (Table 1) were taken from meals served in 53 different restaurants located in 9 different districts of the city of Madrid: Arganzuela, Carabanchel, Centro, Chamartín, Chamberí, Latina, Moncloa, Salamanca and Tetuán. The average apartment price (Euro per square meter) of each of these districts was employed as an indicator of the district's economic level. Average apartment prices were obtained from the Madrid city council (http://www.madrid.es; accession date: 18 July 2018).

Employed fish samples included 17 different species or genera, depending on menu label. The number of samples per restaurant ranged between 1 and 7. Anonymous people acted as clients in those restaurants during the years 2017 and 2018 and sampled tissue from served dishes, including fresh fish, tataki, tartar, carpaccio, ceviche, sashimi, and coated and/or fried fish. The restaurant, the fish name appearing in the menu and the sampling date were annotated. Samples were put into Eppendorf tubes and stored frozen until laboratory analyses.

2.2. Genetic analyses

Genomic DNA was extracted from the samples using DNeasy Blood & Tissue Kit (Qiagen; Verlo, Netherlands). A primer pair amplifying a short fragment (75–125 basepairs, approximately) of the 16S rDNA was employed for DNA amplification. This gene has been demonstrated to allow for reliable species authentication, even in highly degraded samples (Horreo, Ardura, Pola, Martinez, & Garcia-Vazquez, 2012; Muñoz-Colmenero, Martinez, Roca, & Garcia-Vazquez, 2016), and in canned pet food (Armani et al., 2015). Given that the real processing of the samples (from the supplier until it appears on the dish) collected for this study is unknown, and given that the effective processing may not coincide with what is written on the menus, we used this fragment for species identification. The amplification of this fragment assures that the used genetic tools are highly sensitive, and that they will detect species or traces of species present in dishes with a high probability. Consequently, primers 16S-HF and 16S-HR were used. PCR conditions included a total volume of 25 μL containing 1 μL DNA, 12.5 μL DreamTaq Master Mix Polimerase (Thermo Fisher Scientific; Beverly, USA), 1 μL of each primer (10μM) and 9.5 μL of water. PCR cycles included initial denaturation at 94 °C for 4 min, 35 cycles of denaturation at 94 °C for 45s with an annealing at 49 °C for 60s and an extension at 72 °C for 60s, and a final extension at 72 °C for 10 min. PCR products were then purified and finally run on an ABI 3100 sequencer (Applied Biosystems; Foster city, USA). DNA sequences were viewed and edited with the BioEdit alignment editor software (Hall, 1999).

2.3. Species identification

The European Regulation (EU) 1379/2013 establishes which information needs to be declared when selling seafood at the retailer or mass caterer. Each EU Member State has to draft an official list with the trade names (including scientific, local or regional names). These names are the names that need be officially used as product labels in the entire country. The Spanish Ministry of Agriculture, Food and Environment published this list on April 10th, 2014 (BOE-A-2014-3865). The names on this list were employed in this work in order to determine the scientific name that corresponds to each of the menu labels (Table 1). All menu labels detected in this study existed on the official list published by the Spanish Government, only with the exception of “pez mantequilla” (samples S18 and S19), which did not appear in the legislation valid during the samplings (BOE-A-2014-3865). Consequently such samples were deleted from the analyses.

Sequence comparison for species identification was done with the BLAST utility (https://www.ncbi.nlm.nih.gov/BLAST) of the GenBank public database. To this end, we first corroborated that the 16S rDNA sequence of the species indicated on the menu label was present in Genbank (https://www.ncbi.nlm.nih.gov/genbank). Thereafter, BLAST was used to find the most similar sequence. Successful identification existed if the sequence amplified from the collected tissue exhibited a similarity of 100% with a sequence present in GenBank, following Armani et al. (2015). If the most similar sequence rendered by BLAST corresponded to a species/genera that did not coincide with the species/genera mentioned on the menu, we classified the sample as being mislabeled. In these cases, an additional BLAST comparison was done with the DNA sequence of the sample *vs.* the DNA sequence of the taxa mentioned on the menu to test for the robustness of our classification.

2.4. Economic level of Madrid districts

We tested whether there was a correlation between the average apartment price and the percentage of mislabeling. Only districts with two or more samples were included in the Spearman's rank correlation.

3. Results and discussion

All fish samples were successfully amplified. Fragment sizes ranged between 76 and 122 base pairs, which was enough to identify mislabeling on the species/genera level mentioned on the restaurant's menu (Table 1), demonstrating the usefulness of the employed primers even in cooked and processed food. In total, 32 samples exhibited a similarity of 100% with the species mentioned on the menu, and mislabeling appeared in 28.12% of the these samples and in 37.5% of the restaurants. Moreover, using Kappel and Schröder (2016) 2% cut-off threshold (i.e. a sequence identity of ≥98%), mislabeling appeared in 36% of the samples. The here detected proportions of mislabeling do not differ among used thresholds ($\chi^2 = 0.62$, $P = 0.43$) and they are similar to the percentage recently found in Metro Vancouver, Canada (29%; Hu, Huang, Hanner, Levin, & Lu, 2018). Moreover, it is within the range found in European mass caterings (Pardo et al., 2018). The high proportion of mislabeling demonstrates that a more effective control and/or management is required in order to avoid mislabeling and fraud. This is especially important, since among other reasons mislabeling exists for economic issues, and since it can produce health and conservation problems (Everstine et al., 2013; Pramod et al., 2014; Triantafyllidis et al., 2010).

The detected amount of mislabeling is conservative for several reasons. First, we sampled all kind of fish, although one would predict that fraud and thus mislabeling would be most prevalent in expensive species that are difficult to get, e.g. in *Epinephelus marginatus*. Previous