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Biodiversity defrosted: unveiling non-compliant fish trade in ethnic food stores

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ABSTRACT

Out of nearly 30,000 teleosts dwelling in our planet's water bodies, only hundreds of them are commercially exploited and prevail on the global food market. Yet, our estimates of the species actually underpinning global trade is severely hampered by inaccuracy and non-compliance in labelling and reporting. Here, we target ethnic food stores in two British cities (Liverpool and Manchester metropolitan areas), whose numbers are increasing throughout Europe, to examine accuracy of traceability information available to consumers. Despite the existence of thorough EU labelling regulations, we unveil a high level of non-compliance, with a diverse range of poorly-known fish species, often sold without any label or with erroneous information, as demonstrated by DNA barcoding. Results indicate that about 41% of the samples were mislabelled, in stark contrast with a recent study that, in 2015, found < 5% mislabelling in EU supermarkets and fishmongers. These results highlight that inspectors and governments might not be fully aware of the wide diversity of fish species traded, indicating the need for a stronger enforcement of the EU labelling legislations. Compliance with regulations is required not only to protect consumers, but also fish stocks, as for many of the species identified in this survey, population assessment is poor or lacking altogether.

1. Introduction

Global fish production has grown steadily in the last five decades, with fish food supply increasing at an average annual rate of 3.2% (FAO, 2014). World per capita fish consumption increased from an average of 9.9 kg in the 1960s to 19.7 kg in 2013 with preliminary estimates for 2014 and 2015 pointing towards a further growth beyond 20 kg (FAO, 2016). This remarkable development is mainly a consequence of the global population growth expected to reach 9 billion people by 2050 (FAO, 2016). The need to feed this increasing number of people asking for protein sources has driven the rapid growth of the aquaculture sector, which, for the first time in 2014, overtook wild-caught fish production (FAO, 2016). China has played a major role in this growth as it represents > 60% of world aquaculture production (FAO, 2016).

This notwithstanding, half of the seafood consumed by humans still depends on the capture of wild organisms, which amounts to the vast majority of the 1200 species commercialised in the European Union (EU; EUMOFA, 2016) mainly imported as frozen or prepared meals (EUMOFA, 2016). Seafish (SEAFISH, 2015) reports that 70% of the seafood that enters the UK supply chain is imported from abroad or landed by foreign ships. In 2015 UK imported seafood accounted for 5% of the global EU trade. In terms of value, the top UK import species are *Gadus spp*. (cod), *Salmonidae spp*. (mostly farmed Atlantic salmon), *Thunnus* spp. (tuna), *Melanogrammus aeglefinus* (haddock), *Pollachius pollachius* (pollack) and *Scomber* spp. (mackerel).

Data from the retail sector, gathered in 2014, demonstrate that British people preferred to buy frozen seafood (5729 tonnes of the overall seafood sold) as opposed to fresh products (1082 tonnes) or canned seafood (43 tonnes) (Seafood Industry Factsheet, 2015). The increasing demand for frozen seafood, which to a large extent is marketed filleted, beheaded and/or further processed (dried, pre-cooked), makes species identification more difficult. Furthermore, the growth of multiculturalism of Western societies has led to an increase of alternative food stores that trade a wide range of ethnic products (Lee et al., 2013; Armani et al., 2015), many of which purvey a wide assortment of imported seafood products.

Ethnic food stores are often characterized by deficiencies in

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traceability systems and, as a consequence, mislabelling can be a significant issue (Armani et al., 2013; D'Amico et al., 2014; Armani et al., 2015). Seafood is at particular risk, due to the increased globalisation of the trade, the increased imports of newly-exploited and exotic species (Armani et al., 2015; Watson et al., 2015) and the lack of knowledge of seafood products by the average consumer (Velasco et al., 2016). Morphological identification of seafood remains arduous for filleted samples or even for whole, but unusual, newly-marketed species, which would require identification by expert fish taxonomists. DNA-based techniques are currently considered as the gold standard for species identification, in particular through the universal mtDNA COI barcoding fragment (Ward et al., 2005) and a variety of mini-barcodes (e.g. Leray et al., 2013).

In this study, we applied this approach for the identification of frozen fish collected from ethnic retailers in the British cities of Manchester and Liverpool. Food labelling is essential to ensure consumer safety and choice awareness. Considering the recently improved legislation (EC, 2013), which requires seafood to be labelled with commercial and scientific name, production method, catch area and fishing gear category, the mainstream EU retail sector appears to have a stronghold over seafood trade malpractice (Mariani et al., 2015). However, while the main retail sector typically hinges on a handful of commonly traded fish species, ethnic stores purvey small quantities of a much greater spectrum of species caught and farmed worldwide, for which EU Member States have to draw up a list of the commercial designations that are consistently acceptable for specific taxa (i.e. species, genera and, in some cases, entire families). Commercial names permitted in the UK are provided in a governmental publication, "Commercial Designations of Fish" (DEFRA, 2013), which is updated every few years. The scientific name should be in accordance with the FishBase Global Information System on Fish or the Aquatic Sciences and Fisheries Information System database of the Food and Agriculture Organisation.

The main goals of this study were: i) to provide a realistic picture of global biodiversity underpinning the ethnic seafood retail sector in Britain; ii) to verify if the greater diversity of traded species and the lesser profile of the sector would result in high levels of seafood mislabelling; iii) to examine the environmental consequences of poor labelling and traceability of marketed species.

2. Materials and methods

2.1. Samples collection

A total of 88 frozen fish were sampled in 21 different retailers between Liverpool (43 specimens) and Manchester (38 specimens). The final sample size ($N = 88$) of our study is same order of magnitude of comparable investigations carried out in Italy (Armani et al., 2015; D'Amico et al., 2014). Furthermore, during sample collection, we reached a point where it was difficult to locate new stores or find new species that had not already been sampled, therefore reaching a sort of "retail type/product" saturation. Samples were collected in Asian and Afro-Caribbean food shops located mainly in the China town areas of those cities or in Manchester's "Curry Mile" area.

Frozen fish samples ranging from fillets to the whole animal (Fig.1), were gathered between October 2014 and December 2015, trying to maximise the diversity of fish on sale, and focusing on those that did not use standard packaging (e.g. wrapped in a plastic bag, piled in a large freezer with labels hand-written with marker pen, etc.). Samples included wild caught or farmed fish and some were processed (e.g. dried or pre-cooked).

Once collected, samples were dissected in order to remove a little piece of tissue (from muscle or from the caudal fin) suitable for the subsequent genetic analyses.

Tissues samples were placed into 2 ml labelled tubes filled with 95% ethanol and stored at − 20 °C. Details of each sample were collected, including place of purchase, species designation, standard body length (without caudal fin), total length, sex (if the animal was not gutted) and a photograph.

2.2. Molecular analysis

Total DNA was extracted following the standard protocol of Estoup et al. (1996), using Chelex® resin. Tubes containing DNA suspension were then stored at − 20 °C for long-term preservation.

The amplification of the partial COI gene was carried out using the FishF2 and FishR2 universal primers described by Ward et al. (2005). PCR reactions were performed in a total volume of 20 μl following a protocol by Serra-Pereira et al. (2010). Each amplification contained: 2 μl 10 × reaction buffer, 1 μl $MgCl_2$ (50 mM), 0.2 μl of each primer (0.01 mM), 0.1 Units of DNA Taq Polymerase (PROMEGA, Madison, WI, USA) and 0.4 μl dNTP (10 mM). PCR conditions entailed an initial denaturating step at 94 °C for 2 min, then 35 cycles of denaturation at 94 °C for 30 s, annealing at 52 °C for 40 s and extension at 72 °C for 1 min followed by a final extension at 72 °C lasting 10 min. If amplifications were unsuccessful with the FishF2 and FishR2 primers due to low DNA quality, COI mini-barcode primers (mICOIintF and jgHCO2198) were used following the protocol described in Leray et al. (2013). PCR products were visualized on 1% agarose gels with 6 μl of GelRed by means of ultraviolet transilluminator. Amplicons were sequenced by Source Bioscience Sequencing Service (Cambridge, UK) using the forward primer. Sequences quality was checked by eye using the chromatogram visualization software BioEdit v7.2.5 (Hall, 1999). Samples were identified using two online databases, 1) the GenBank database (http://www.ncbi.nlm.nih.gov/) and 2) the Barcode of Life Data system (BOLD, http://boldsystems.org/; Ratnasingham and Hebert, 2007). The "Public Record Barcode Database" was used in the latter case, where identification was determined by sequence similarity to the reference database (Wong and Hanner, 2008) and checked by "Tree based identification" (Costa et al., 2012).

The BLAST platform allows the assignment of a DNA sequence to a species by means of sequence comparison with database entries. However for an accurate identification, the *E*-value, as an evidence of error probability, should go as far as possible to zero and the sequence match should be ≥ 98% identity.

Lastly, in order to assess the reliability of the sequences, each matching sequence was aligned with our unknown sequence using the Clustal W alignment algorithm in BioEdit.

Statistical analysis of the results present in this study show 95% confidence intervals for binomial distribution and were carried out using MASS package (Venables and Ripley, 2002) within the statistical software R (version 3.3.3, R Development Core Team, 2017).

2.3. Determination of labelling accuracy and substitutions

Samples labelling accuracy was checked against the European legislation EU no 404/2011 further implemented with the EC No 1379/2013, which relates to consumers' information and labelling provisions for fishery and aquaculture products marketed within the Community. These products, irrespective of their origins, must be appropriately labelled at the point of the retail, reporting the scientific name, the commercial designation, the production method (caught at sea or inland waters or farmed), the catch area and the fishing gear used.

In order to confirm whether substitutions occurred within our dataset, the species IDs obtained via molecular analysis were checked against the official DEFRA list of seafood product denominations (DEFRA, 2013).

3. Results

Based on the requirements of the most recent EU labelling regulation (EC No 1379/2013), none of the samples provided comprehensive