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Mislabeling in seafood products sold on the Italian market: a systematic review and meta-analysis.

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Abstract

In this study the results of a systematic review and meta-analysis on mislabeling in seafood products sold on the Italian market ~~is~~ are presented~~.~~ The aim was especially targeted to answer the research question “What is the mislabeling rate ~~occurring at national level~~ in seafood products sold on the Italian market?”. Scientific papers (SPs), were filtered using pre-determined inclusion criteria and data related to sampling and mislabeling was analyzed. ~~No time limit was set, and the search was concluded in June 2022~~. Samples were categorized according to their taxon (species, family order) or generic market group (MG), market form (unprocessed/processed), distribution channel and geographical area. Samples were considered mislabeled when the species found by molecular analysis did not comply the information indicated in the label. The mislabeling rate (m. r.) was weighted on the sample size and provided overall and for each category. In the 51 selected SPs (published from 2005 to 2022) the most sampled taxa were fish (83.8%): mackerels, cods, herrings, flatfishes and jacks were the most represented. Unprocessed fillet/slice was the most analyzed market form (61.4%), and samples were especially collected at retails (76.5%). Ten regions were sampled, especially Tuscany and Apulia. The overall weighted m. r. was 28.4% (CI 26%-30%), falling within the m. r. range found at international level (Luque & Donlan, 2019). M. r. over the CI (>30%) were observed in 1) jellyfishes, European perch, European grouper, Atlantic mackerel and samples labelled as “*spinarolo*”, “*baccalà*” or “*palombo*”; 2) Unprocessed fresh, processed salted and highly processed samples; 3) small distribution channel; 4) Southern regions. Significative differences in m. r. concerned taxa, distribution channels and geographical areas. Despite some bias of the SPs may affect the results (lack of sampling plans; poor data on molluscs and crustaceans; no standardization in m. r. interpretation) this is the first systematic review and meta-analysis that, synthesizing evidence ~~providing an accurate characterization~~ on ~~of~~ Italian seafood mislabeling, can support ~~can direct~~ policy making ~~official control activities~~ for minimizing frauds impacts.

Keywords

Food Frauds, DNA analysis, species substitution, labeling compliance, risk assessment.

1. Introduction

In EU Member States, food frauds increased by 85% between 2016 and 2019, and it is expected that the percentage further rise (Visciano & Schirone, 2021). For these reasons, the EU has placed increasing emphasis on the prevention of deceptive practices, and the Regulation (EU) No 2017/625 came into force updating agri-food chain control policies and reinforcing protection of consumers against frauds. Also, the definition of food fraud provided by the European Commission was therefore recently revised in the light to the aforesaid Reg. as "*any suspected intentional action by businesses or individuals for the purpose of deceiving purchasers and gaining undue advantage therefrom, in violation of the rules referred to in Article 1(2) of Regulation (EU) 2017/625* (European Commission, 2018). Given the extent of the phenomenon, 'food fraud notification' has been also included in the Rapid Alert System for Food and Feed portal (iRASFF) (Commission Implementing Regulation EU No 2019/1715).

The European Parliament identified seafood as the second-highest food category at risk for fraud (Kroetz et al., 2020) due to the globalization of supply chains and the introduction of increasingly complex distribution systems. While seafood fraud comes in a variety of forms, mislabeling, meaning "*false claims or distortion of the information reported on the label*" (European Commission, 2018), is perhaps the most concerning (Kroetz et al., 2020; Reilly, 2018; Van Holt, Weisman, Käll, Crona, & Vergara, 2018). Although mislabeling may be unintentional – for instance when several species are handled on the same manufacturing equipment –in most cases it disguises illegal practices that are carried out for financial gain at every stage of the marketing chain (Reilly, 2018). Mislabeling involves the intentional substitutions of high-quality species with less expensive varieties, or farmed versus wild sourcing, or even the selling of fish from Illegal, Unreported and Unregulated (IUU) fishing and the recycling of by-catches or fish waste (Helyar et al., 2014; Hu, Huang, Hanner, Levin, & Lu, 2018; Kroetz et al., 2020; Reilly, 2018). Potential consequences include economic losses, ecological impact, undermining of sustainability efforts and, considering that food labeling is the most important instrument for consumer decision-making and food choice, disrespecting of consumers' religious or ethical reasons. In addition, the illicit presence of toxic species (Giusti et al., 2018) or the omission of ingredients potentially causing allergies (e. g. crustaceans or molluscs) may lead to human health risks (Luque & Donlan, 2019; Pardo, Jiménez, & Pérez-Villarreal, 2016).

Therefore, besides the principles of the General Food Law (Regulation EC No 178/2002) and the general provision of food information to consumers (Regulation EU No 1169/2011), specific provisions for the labeling of fishery and aquaculture products were established by the Regulation (EU) No 1379/2013. This Regulation imposes to the Member States to publish a list reporting the official seafood trade names, corresponding to species scientific names, accepted within the national territories. Yet, even where seafood traceability regulations are progressive, mislabeling continues to be documented (Luque & Donlan, 2019).

With the development of molecular tools and specifically DNA-testing, also proposed by Regulation (EU) No 1379/2013 to deter operators from falsely labeling catches, studies investigating seafood mislabeling have increased substantially. In many cases, these studies investigated a particular product, geography, or a specific stage within the supply chain. On the contrary, few reviews on mislabeling have been published in the last decade. In addition, most of them tend to be mainly descriptive and do not based on a systematic approach (Golden & Warner, 2014; Pardo et al., 2016; Warner, Mustain, Lowell, Geren, & Talmage, 2016). Systematic reviews, increasingly popular in many other scientific research fields, involve in fact a detailed and comprehensive plan and literature search strategy derived *a priori*, with the goal of identifying all relevant studies on a particular topic (Petticrew & Roberts, 2006; Uman, 2011), and often include a meta-analysis component using statistical techniques to analyze the data from several studies (Petticrew & Roberts, 2006; Mikolajewicz & Komarova, 2019). This kind of literature revision was rarely applied for investigating mislabeling in seafood. Recently, Luque and Donlan published two systematic reviews associated with meta-analysis to characterize global seafood mislabeling (Luque & Donlan, 2019) and exploring its causes using price data from mislabeling studies (Donlan & Luque, 2019). The same approach was then used by Blanco-Fernandez et al. (2021), but only to analyze mislabeling trends in hakes during the last 17 years. In these systematic reviews, outcomes from data analysis were aggregated for more countries (Blanco-Fernandez et al., 2021; Donlan & Luque, 2019; Luque & Donlan, 2019). Therefore, the estimation of the global mislabeling rate may be distorted by the different approach adopted across countries for the definition of the official seafood list at national level. In fact, the "one species one name approach" proposed by Lowell, Mustain, Ortenzi, & Warner (2015) is not always applied and the association between the scientific name and the correspondent trade name greatly varies among countries, even within European territory. For instance, the trade name "anchovy", that in Italy is univocally associated to *Engraulis encrasicolus* (Italian trade name "*acciuga*" or "*alice*") (Ministerial Decree n. 19105 of September the 22nd, 2017), is instead associated to all the species of the Family Engraulidae in UK (United Kingdom Department for Environment, Food and Rural Affairs, 2013). Therefore, mislabeling interpretation for anchovy-based product is different between these two countries.

In the meta-analysis ~~systematic review~~ performed by Luque & Donlan (2019) it was observed that Italy, together with Spain and US, was the country with the largest number of studies on this topic. Despite this, to the best of our knowledge, no reviews have been performed yet in any EU country. Since Italy is included in the four EU countries with highest seafood consumption (EUMOFA, 2021), an accurate characterization of seafood mislabeling in the national market is crucial to assess the causes and consequences of this practice, and to design solutions to reduce it. In fact, a better understanding of the scale and nature of seafood mislabeling is important for improving ~~regulatory efforts~~making policy and consumer engagement programs aimed at minimizing its societal costs (Kroetz, Donlan, Cole, Gephart, & Lee, 2018).

In this study a systematic review and meta-analysis was performed to document mislabeling occurrence in seafood products sold on the Italian market. The aim was especially targeted to answer the research question "What is the mislabeling rate in seafood products sold on the Italian market?"

~~We aim to answer the question "What is the mislabeling rate occurring at national level?"~~ by providing an evidence synthesis of all the research performed on this topic.~~to document mislabeling occurrence in seafood products sold on the Italian market was performed~~. Since our review only included studies performed in Italy, it counted on a specific regulatory framework represented by the Italian lists of seafood trade names reported by the Ministerial Decrees of 2002, 2008 and 2017 (the last the one currently in force). Outcomes from this study could also provide a risk assessment according to seafood species, market form, distribution channels and geographical area of collection and could serve for driving more targeted official control activities. Finally, by highlighting and discussing strengths and shortcomings arising from data analysis, this study ~~review~~ can serve to improve the inquiry approach in this research area.

2. Materials and Methods

2.1 Bibliographic search and scientific papers collection

The bibliographic search was carried out on three scientific databases (Google Scholar, PubMed and Web of Science) using the keywords "seafood AND (species identification OR DNA OR molecular) AND Italian market AND mislabeling". The relevance of the retrieved scientific papers (SPs) was assessed based on the title and of the abstract. To make the SPs collection as complete as possible, a snowball search was conducted checking the reference lists of the selected articles, and Google Scholar "cited by" function was also used. No time limit was set, and the search was concluded in June 2022. After deduplication, SPs were considered eligible and included in the study only if 1) represented by peer-reviewed studies (quality assurance); 2) molecular techniques based on DNA analysis (e. g. DNA barcoding or metabarcoding, phylogenetic analysis, multiplex PCR, RFLP-PCR, etc.) were used for species identification; 3) the analysed samples belonged to seafood products sold on the Italian market; 4) the seafood products sample sizes was reported; 5) the seafood products reported information on the generic taxon (fish, mollusc, crustacean, other) and /or trade name (generic or specific, e. g. tuna or Yellowfin tuna) and/or scientific name of the species on the label (e. g. *Thunnus albacares*). In fact, this latter information represents the minimum criteria to make a comparison between the label declaration of the samples (from now defined as "blind samples" – "*bs"*) and the molecular results. In the case of SPs also analysing samples belonging to morphologically identified reference samples (e. g. specimens of known species directly purchased on the market or specially provided by fishermen, research institutes, national Competent Authorities, etc.), only the *bs* were included in the analysis, since the reference samples do not fulfil the scope of the study. On the finally included SPs, information on the years of publication, scientific journals and corresponding author/s affiliation were analysed. Then, information on sampling, namely *bs* number, taxonomic information reported on the label, processing degree, distribution channels and geographical area of collection and data on mislabeling cases were recorded, when reported. All data were registered in an Excel file and analysed as reported in section 2.2. Information on the molecular technique used for species identification will be included in another more technical paper.

2.2 Analysis of data related to sampling

The *bs* number overall, for each SP and year of publication was calculated. Since most of the SPs did not specify the *bs* collection year/s, we decided to use the SP year of publication to standardize the analysis. Respect to the data relative to taxon, market form and distribution channel, we categorized the *bs* according to definition provided by legislation and official reports. In particular, the taxon (order, family and species) was assigned in accordance with the FAO FishBase/SealifeBase Information System as reported by Regulation (EU) No 1379/2013; the market form was assigned according to the definition of the Regulation (EC) No 852/2004 and based on the European Market Observatory for Fisheries and Aquaculture Products report by EUMOFA (2021); for the distribution channel assignment, the definition provided by the Regulation (EU) No 1169/2011 and Regulation (EU) No 1379/2013 were used. Then, the recorded data were analysed as described in the following sections.

2.2.1 Taxonomic information reported on the bs label. If a specific trade name (in English) that can be unequivocally associated to a single species according to FishBase (https://www.fishbase.se/search.php) or SealifeBase (https://www.sealifebase.ca/), presented as taxonomic reference sources under Regulation (EU) No. 1379/2013, was declared in the label, the species scientific name was recorded (e. g. "Yellowfin tuna" was recorded as *Thunnus albacares*). Likewise, considering that the trade name of the *bs* were often translated in English from Italian in the analyzed SPs, the Italian official lists of seafood trade names were also in some specific cases consulted. For this purpose, the official list in force in the year of the SP publication was used (Ministerial Decree of March 27th, 2002; Ministerial Decree of January 31, 2008; Ministerial Decree n. 19105 of September the 22nd, 2017). For instance, if the generic trade name "hake", that in Fishbase is associated to several species, was reported in a *bs* from a SP performed in 2020, it was assumed that, if not differently specified in the SP, it corresponds to the Italian designation *"nasello* or *merluzzo*" (Merluccidae), reported in the Ministerial Decree n. 19105 of September the 22nd, 2017, which is in force since September 2018. After this preliminary step, the *bs* was first categorized in fish, molluscs (cephalopods or bivalves), crustaceans, and other different taxa and then associated to an order (highest taxonomic rank level) according to FishBase and SealifeBase. For instance, if a label declared "Tuna" or "Yellowfin tuna" or "*Thunnus albacares*", the *bs* was associated to the order Scombriformes. To simplify the reading for non-expert taxonomists, the order was associated to a generic market group (MG) according to FishBase and SealifeBase (such as "mackerels" in the case of the order Scombriformes). Finally, the *bs* within each MG were further organized into lower taxonomic levels (family and species). Obsolete nomenclatures were substituted with valid ones, according to FishBase and SealifeBase. The *bs* number for each MG, family and species was provided.

2.2.2 Bs market form. The *bs* were categorized based on their market form in unprocessed and processed according to the definition of the Regulation EC No 852/2004 and EUMOFA, 2021 report; unprocessed *bs* were divided in fresh or frozen, while processed *bs* were further categorized in salted, dried, breaded, smoked, canned (in oil, in sauce, in water, marinated, fermented, pastes), pre-cooked or cooked (fried, baked, boiled) and highly processed seafood preparation (burger, minced, balls, cakes, filling, surimi, etc.). The *bs* number for each market form was provided overall and divided for species, when possible.

2.2.3 Bs distribution channels. For the distribution channels, it was considered "small distribution" if local retails, fish markets, fish shops, fishmongers, groceries, ethnic shops or other small retailers were involved; "large distribution" in the case of supermarkets, hypermarkets, wholesalers, department stores, fish companies and other large-scale retailer markets; "mass caterers" which includes food businesses such as restaurants, canteens, schools, hospitals and mass caterer enterprises (Regulation EU No 1379/2013); additionally, although not properly involving the *bs* distribution channels, sampling that were performed in the context of "official control" activities were considered, for instances if the *bs* were provided by Border Control Points (BIPs), Port Authorities, Local Health Authorities (LHAs) or anti-adulteration and health unit (NAS). The *bs* collected by official control authorities for only research purposes were also included in this category. The *bs* number for each distribution channel was provided overall and divided for species, when possible.

2.2.4 Bs geographical area of collection. The geographical areas of *bs* collection were categorized in Northern Italy (Valle d'Aosta, Piemonte, Lombardia, Trentino-Alto Adige, Veneto, Friuli-Venezia Giulia, Liguria and Emilia-Romagna), Central Italy (Latium, Marche, Tuscany and Umbria) and Southern Italy (Abruzzo, Basilicata, Calabria, Campania, Molise and Apulia) and Islands (Sicily and Sardinia). The *bs* number for each geographical area, with details on the region, was provided overall and divided for species, when possible.

2.3 Analysis of data related to mislabeling

2.3.1 Mislabeling rate calculation. In the collected literature, mislabeling was usually described as the non-compliance between the species identified by molecular analysis and the trade name/scientific name declared on the product label. However, it was sometimes referred to the lack of one or more labeling information required by legislation. In this study, the *bs* were considered mislabeled only in the first case, when the species found by molecular analysis did not comply the information indicated in the *bs* label. Thus, the formula [(*bs* number showing non-compliance between species identified by molecular analysis and information reported on the label/*bs* number *100] was applied to calculate the mislabeling rate (m. r.). We excluded from the m. r. calculation the *bs* reported by the source as not identified to any level of taxonomy (the outcomes on mislabeling rates were in some cases normalized accordingly). The overall m. r. was weighted based on the sample size of the study, and the relative confidence interval (C.I.) was calculated. The m. r. was also reported for each publication year, taxon, market form, distribution channel and geographical area. Initially, we decide to include the SPs regardless of their *bs* number, also considering that in some cases this information was modified for the study purpose. Despite this, we think that a minimum number of samples is essential to represent the market scenario. Therefore, we only considered data on m. r. for which a number of *bs* $\geq$30 was investigated. To facilitate the discussion, they were categorized according to their distance from the C. I. of the overall weighted m. r. in 1) category A (over the upper value of the CI); 2) category B (within the CI); 3) category C (under the lower value of the CI). Significant results were considered as those associated with $p < 0.05$. If overall significance was observed, pair-wise comparisons were analyzed using $\chi 2$ test. We defined as "expected species" (or higher taxonomic level), the species supposed to be present in the product based on the *bs* label and as "substitute species" (or higher taxonomic level), the true identity of a mislabeled *bs*. We searched "substitute species" against the IUCN Red List of Threatened Species database (https://www.iucnredlist.org/) to assess their conservation status and the relative ecologic impact of mislabeling.

3. Results

3.1 Bibliographic search and scientific papers collection

Overall, 51 SPs were finally selected (Table 1), published from 2005 to 2022 (except for 2006). ~~In general, t~~The number of SPs has started to increase since 2015 in which, also,~~.~~ t~~T~~he highest number of SPs was published ~~in 2015~~ (7 SPs)~~, followed by 2019 (5 SPs) and 2016, 2017, 2018 and 2020 (4 SPs each). From 1 to 3 SPs were published in the other years~~ (Table 1). Many of the selected SPs (n=17; 33.3%) were published in "*Food Control*", followed by "*Foods*" (n=4; 7.8%) and "*Food*

Research International", "*Journal of Agricultural and Food Chemistry*" and "*Italian Journal of Food Safety*" (3 SPs each; 5.9% each). One or 2 SPs were distributed in other 17 international journals. Overall, researchers from the Departments of Veterinary Sciences were the most involved. The SPs corresponding authors were in fact affiliated to the Department of Veterinary Sciences of the University of Pisa in 13 cases (25.5%), followed by Department of Biological, Geological and Environmental Sciences of the University of Catania (n=9; 17.6%) and the Department of Veterinary Sciences of the University of Bari (n=8; 15.7%). Other 17 affiliations were found in the other 21 SPs (1-3 SPs for each affiliation) (Table 1).

3.2 Analysis of data related to sampling

3.2.1 Bs number (overall, for each SP and year of publication). Overall, 3576 *bs* were analysed in the 51 SPs, ranging from 3 to 290 (Table 1). The *bs* number was distributed in the years as reported in Figure 1, and it was generally in line with the number of published SPs;, with the highest *bs* number was in fact in 2015 (565 *bs*; 15.8%), 2019 (528 *bs*; 14.8%) and 2017 (489 *bs*; 13.7%).

3.2.2 Taxonomic information reported on the bs labels. Overall, most of the *bs* belonged to fish (2997 *bs*; 83.8%); mollusc accounted for 360 *bs* (10.1%), of which 263 cephalopods, 95 bivalves and 2 not specified, and crustacean accounted for 82 *bs* (2.3%). Additionally, 56 *bs* (1.6%) belonged to Cnidaria (jellyfish) and 1 *bs* (0.03%) to Amphibia (frog). Finally, 14 *bs* (0.4%) were labelled with names referable to vegetables (namely bamboo, mustard tuber, lily flower), even though morphologically recognized by the authors as jellyfish-based products (Armani et al., 2013). The remained 66 *bs* were composed by mixture of fish and molluscs (53 *bs*; 1.5%), molluscs and crustaceans (10 *bs*; 0.3%) and fish, molluscs and crustaceans (3 *bs*; 0.1%). To note that few SPs specifically investigated seafood categories different from fish (n=4; 7.8%), and, usually, non-fish *bs* were included in more extensive SPs, where also fish were analysed.

It was possible to allocate 3390 *bs* to 49 MG (based on the order association found in FishBase and SealifeBase), of which 5 mixed together (e. g. Porgies/Temperate Basses) (Table SM1), while 186 *bs* were not allocated to any MG as their label only reported the term fish, molluscs, crustaceans

~~(or their mix) and vegetables~~. The association between the MG with the respective order is specified in Table SM1.~~ To be noted that in the case of the orders Eupercaria *incertae sedis* and Carangaria *incertae sedis*, the MG used by FishBase to characterize the families instead of the orders was used, given the high number of families included in these orders~~. Thus, respect to the MG, mackerels with 543 *bs* (16.0%), and cods with 513 *bs* (15.1%) were the most numerous collected *bs*, followed by herrings (326 *bs;* 9.6%), flatfishes (313 *bs;* 9.2%), and jacks (195 *bs;* 5.8%).

To consider that *bs* belonging to cods were collected during almost all the considered period (14 out of 17 years),~~ with the highest number in 2016 (176 *bs*; 34.3% of cods) and 2013 (110 *bs*; 21.4%)~~; The collection of *bs* belonging to mackerels was performed during 9 years,~~ and especially concentrated in 2010 (233 *bs*; 42.9% of mackerels), 2019 (101 *bs*; 18.6%) and 2017 (76 *bs*; 14.0%)~~. The *bs* belonging to herrings, flatfishes and jacks were concentrated in 11 (herrings) or 10 (flatfishes and jacks) years; ~~herrings were especially collected in 2019 (233 *bs*; 71.5% of herrings) and 2015 (61 *bs*; 18.7%); flatfishes in 2019 (106 *bs*; 33.9% of flatfishes) and 2015 (95; 30.4%) and jacks in 2015 (80 *bs*; 41.0% of jacks)~~ (Figure 2). In all the years, the number of SPs ranged from 1 to 2, except for cods in 2016 (4 SPs), mackerels in 2018 and 2021, and flatfishes in 2018, with 3 SPs each.

Respect to the lower taxonomic levels, 3183 (93.9%) and 2521 *bs* (74.4%) out of 3390 *bs* assigned to MG were allocated to a unique family and to a unique species, respectively (all listed in Table SM1). Overall, 75 families (53 belonging to fish, 9 to molluscs, 12 to crustaceans, and 1 to cnidaria) and 219 species (148 belonging to fish, 46 to molluscs, 24 to crustaceans, and 1 to cnidaria) were found. Among families, Scombridae (540 *bs* out of 3183 *bs* assigned to a unique family; 17.0%) were the most numerous, followed by Gadidae (303 *bs*; 9.5%), Merluccidae (201 *bs*; 6.3%), Xiphiidae (190 *bs;* 6.0%), Engraulidae (182 *bs*; 5.7%), Clupeidae (144 *bs*; 4.5%), and Pleuronectidae (121 *bs;* 3.8%). Respect to the species, 276 out of the 2521 *bs* assigned to a unique species were declared as *T. albacares* (10.9%), followed by *Xiphias gladius* (160 *bs*; 6.3%), *Clupea harengus* (127 *bs*; 5.0%), *E. encrasicolus* (119 *bs*; 4.7%), *Pleuronectes platessa* (97 *bs*: 3.8%), *Perca fluviatilis* (94 *bs*; 3.7%) and *Gadus morhua* (90 *bs*; 3.6%). Other MGs, families and species with *bs* number covering percentages

≥ 0.5% are reported in Figure 3. Twenty-three out of the 219 species found in this analysis (10.5%) were not reported in the Ministerial Decree (list of seafood trade names) in force in the respective SP publication year. To highlight that 10 of them have been subsequently included in the list of the Ministerial Decree that was released later, while the remained 13 are not still not reported.

The five more represented associations among MGs, families and species analysis were: 1) MG mackerels, family Scombridae, species *T. albacares;* 2) MG cods, families Gadidae and Merluccidae, species *G. morhua*, *G. chalcogrammus* and *Merluccius merluccius;* 2*;* 3) MG herrings, families Engraulidae and Clupeidae, species *C. harengus* and *E. encrasicolus*; 4) MG flatfishes, family Pleuronectidae, species *P. platessa;* 5) MG jacks, family Xiphiidae, species *X. gladius* (Figure 3; Table SM1).

3.2.3 Data on bs market form. Overall, 2197 out of the total 3576 analyzed *bs* (61.4%) were unprocessed (mainly not whole as fillets or slices) and 1379 *bs* (38.6%) were processed. Among the unprocessed *bs* the percentages of fresh and frozen groups were slightly biased in favor of fresh (36% versus 27%) with a large part of *bs* (37%) for which it was not specified if they were fresh or frozen (Figure 4a). As regards the number of processed *bs*, canned highly exceeded that of the other groups, representing the 43.0% (593 *bs*) of processed *bs* (Figure 4b).

The correlation between species (or higher taxonomic level, when the species was not declared) and market form was made for 3313 *bs* out of the overall 3576 (92.6%)~~ since the SPs by Armani et al. (2015b), Armani et al. (2016) and Cutarelli et al. (2018), in which 68 *bs*, 135 *bs* and 60 *bs* were analyzed, respectively, did not provide this information~~. Considering this, the *bs* number of each market form were modified accordingly (Table SM2). The highest number of unprocessed fresh *bs* were represented by slices of *X. gladius* or swordfish that covered 17.6% (139 *bs*) of unprocessed fresh *bs* considered in this step; ~~among the other main investigated MGs/family/species associations (section 3.2.2), *T. thynnus* (mackerels - Scombridae) was also especially included in unprocessed fresh, together with *M. merluccius* (cods - Merluccidae), and *P. platessa* (flatfishes - Pleuronectidae).~~

The highest number of unprocessed frozen *bs* were represented by *Pangasianodon hypophthalmus* (9.4%). To note that most of unprocessed frozen *bs* made of *P. hypophthalmus* (51 out of 54) were analyzed in the SP by Bellagamba et al. (2015).~~, aimed at identifying this species from other closely related species, rather than assessing mislabeling in this kind of product. However, considering that fillets analyzed were purchased from the market to develop the molecular approach, they were considered as *bs* in this study.~~ Among the other main investigated MGs/family/species associations (section 3.2.2), *G. morhua* and *G. chalcogrammus* (cods - Gadidae) were also often collected as unprocessed frozen fillets. All processed canned *bs* was made of species included in the most investigated MGs/family/species associations (section 3.2.2), especially represented by *T. albacares* or tuna (mackerels, Scombridae) (46.1%; n=243), followed by *E. encrasicolus* or anchovy (26.9%; n=142), *C. harengus* (10.4%; n=55) (herrings – Clupeidae) and *Scomber scombrus*/other *Scombrus* spp. (9.3%; n=49) (mackerels, Scombridae).

Likewise, the processed breaded *bs* were especially composed by species belonging to cods, and the genus *Merluccius* spp. was most represented (60.2 %, n=142). Processed salted *bs* were especially composed by "baccalà" (42%; n=86), intended as *G. macrocephalus* or *G. morhua* (cods - Gadidae) according to the Italian lists of seafood trade names. Processed smoked and highly processed seafood preparations were especially made of *C. harengus* (82.4%; n=70) (herrings – Clupeidae) and *G. chalcogrammus* (41.2%; n=21) (cods - Gadidae), respectively. More details on species (or higher taxonomic level) sampling for each market form are reported in Table SM2.

3.2.4 Data on bs distribution channels. Most of the *bs* were collected at retail level (2736 *bs* out of 3576; 76.5%), of which 876 (32%) performed in large distribution channels and 238 (8.7%) in small distribution channel, while this distinction was not made for the remained 1622 *bs* (59.3%). Of the 238 *bs* from small distribution channel, 144 (60.5%) were collected in ethnic retailers (especially Chinese communities). Additionally, 413 *bs* (11.7%) were collected at mass caterers (4 of which in ethnic restaurants), and 400 *bs* (11.3%) in the context of official control activities (especially from

Border Control Posts, and Porth Authorities). The remained 27 *bs* were collected at small distribution level and from official control activities without further details (Armani et al. 2011).

A wide number of species (or higher taxonomic levels) were collected at retail level (small or large distribution), with *bs* labeled as *T. albacares* or tuna as the most numerous (392 *bs*; 14.3% of 2736 *bs* collected at retail level). The *bs* declared as *R. esculentum* or jellyfish or vegetables ~~(but recognized to be jellyfish-based products by Armani et al., 2013)~~ were the most collected in ethnic retailers (66 *bs*; 45.8% out of 144 herein collected). At mass caterers, *bs* labeled as *T. thynnus* or *Thunnus* spp. or tuna were the most numerous (67 *bs*; 16.2% of 413 *bs* herein collected), followed by salmon (61 *bs*; 14.8%). To be noted that at mass caterers there were more cases of *bs* labeled with no scientific names.~~ All the 4 jellyfish *bs* were collected in ethnic restaurants (Armani et al., 2013)~~. Finally, the *bs* collected in the context of official control activities belonged to various species, with *T. albacares* at the top (68 *bs*; 17.0% of *bs* from official control activities). More details on species (or higher taxonomic level) sampling for distribution channel are reported in Table SM3.

3.2.5 Data on bs geographical area of collection. ~~Respect to t~~The geographical area was detailed only for ~~of the sampling. it was detailed for~~ 2475 *bs* out of 3576 *bs* (69.2%):~~, while the other 1101 (30.8%) were only generically reported as collected on the Italian territory. Thus,~~ 1056 ~~out of 2475~~ *bs* (42.7%) were collected in Southern Italy, 870 *bs* (35.2%) in Central Italy, 345 (13.9%) in Northern Italy and 25 *bs* (1.0%) in Islands. Additionally, 139 *bs* (5.6%) were collected in both Northern and Central Italy and 40 *bs* (1.6%) in both Southern Italy and Islan-ds. Ten regions out of 20 were sampled - Emilia-Romagna, Liguria, Lombardia (Northern Italy), Latium, Marche, Tuscany (Central Italy), Apulia, Calabria, Campania (Southern Italy) and Sicily (Islands) – and the region of the sampling was detailed for 2187 *bs* (88.4% out of the 2475 where the geographical area was reported). Tuscany and Apulia, with 864 *bs* (39.5% out of 2187 *bs*) and 808 *bs* (36.9%), respectively, were the most sampled regions. Apulia (76.5% of *bs* in Southern Italy), Tuscany (96.0%. *bs* in Central Italy) and Emilia-Romagna (21.2% of *bs* in Northern Italy) were the most sampled region of Southern, Central and Northern Italy, respectively. In Southern Italy and Islands (Sicily), *bs* labeled as *X. gladius* or swordfish were the more sampled (15.3% and 60% of *bs* collected in these geographical areas, respectively). *C. harengus* and *E. encrasicolus* or anchovy were especially sampled in Central Italy (13.4% and 6.4%, respectively) and *P. hypophthalmus* in Northern Italy (15.9%). More details on species (or higher taxonomic level) sampling for each geographical area are reported in Table SM4.

3.3 Analysis of data related to mislabeling

3.3.1 Mislabeling rate (m. r.) calculated overall and for year of publication. Overall, 3534 out of the total 3576 investigated *bs* (98.8%) were used to calculate the overall weighted m. r., since *bs* where the taxonomic identity was not achieved (e. g. for technical failures in DNA amplification/sequencing, use of ineffective or poorly discriminating molecular targets, etc.) was not included in the count. Overall, 1005 *bs* were found as mislabeled, with an overall weighted m. r. of 28.4%, (95% CI 26 and 30). Thus, the m. r. found in this study were categorized as follows: A) m. r. >30%; B) m. r. from 26% to 30%; C) m. r. < 26%. Categories A and C were further divided in A1 (m. r. > 50%), A2 (m. r. from >30% to 50%), C1 (m. r. from 10% to <26%) and C2 (m. r. < 10%).

The distribution of the mislabeled *bs* throughout the years with relative m. r. is reported in Table 2. The m. r. was not provided for 2005, 2008, and 2009 since a *bs* number <30 was investigated in these years. The higher m. r. (category A1) was observed in 2013 (61.9%), 2010 (54.1%) and 2015 (49.7%). The m. r. of these three years were significatively higher respect to the other years (p values <0.05). In general, a decreasing trend in m. r. values was observed since 2016, with m. r. calculated for each year included in C category (C1 or C2), except for 2020 (A1) and 2022 (B) (Table 2).

3.3.2 Mislabeling rate calculated for taxon. All the m. r. calculated for taxon (species or higher taxonomic level, when the species was not declared) are reported in Figure 5 and Table 3. Within the category A1, there were found: *Rophilema esculentum* or vegetables (merged with *R. esculentum* as morphologically recognized as jellyfish-based products by Armani et al. 2013) (m. r. 93.1%). This type of products was found as especially substituted with the jellyfish *Nemopilema nomurai* (81% of the cases); *Squalus acanthias* or *S. blainville* (the two species that can be associated to the Italian trade name "*spinarolo*" according to both the Ministerial Decree of January 31, 2008 and Ministerial

Decree n. 19105 of September the 22nd, 2017) (m. r. 86.7%). These species were found substituted with *Prionace glauca* (Italian trade name "*verdesca*") in 100% of the cases; *P. fluviatilis* (m. r. 85.1%), particularly substituted with *P. hypophthalmus* and *Lates niloticus* (46.8% and 31.0% of the cases); *T. thynnus* (m. r. 77.8%), substituted with *T. albacares* in 85.6% of the cases; "Baccalà" (m. r. 67.5%), where the species *G. Macrocephalus* or *G. morhua* were especially substituted with *Pollachius virens* (65.4% of the cases); *Epinephelus marginatus* (m. r. 67.1%), substituted with *L. niloticus* in 76.4% of the cases; *S. scombrus* (m. r. 64.3%), especially substituted with *S. colias* (77.8% of the cases); *M. merluccius* (m. r. 62.7%), especially substituted with *M. productus, M. hubbsi* and *G. chalcogrammus* (28%, 25% and 25% of the cases, respectively); *Mustelus mustelus, M. asterias, M. punctulatus* ("*palombo*") (mislabeling rate 59.6%) especially substituted with *S. acanthias* (41.1%). Particularly low m. r. (category C2) was observed for *Octopus vulgaris* (m. r. 7.3%) and *E. engrasicolus* or anchovy (m. r. 6.4%). Only *Sepia officinalis* and *P. glauca* showed m. r. within the overall m. r. confidence interval (category B), with 26.0% and 26.1%, respectively. Beyond the m. r. reported above, the major number of substitution cases (120 out of 987) was observed for *T. albacares*, linked to the high number of *bs* overall analysed for this species.

Overall, 150 species were found to be used as substitute (Table SM5). By searching them against the IUCN Red List it was found that 11 (7.3%) were "*vulnerable*", 6 (4.0%) "*endangered*" and 2 (1.3%) "*critically endangered*" (Table SM5). Health implications were only highlighted for 2 *bs* labeled as squid but identified as *Lagocephalus* spp., a poisonous pufferfish species banned from the EU market (Armani et al., 2015b). Additionally, two SPs especially highlighted the omission of molluscs in the ingredient list of some surimi-based products (Giusti et al., 2017; Piredda et al., 2022).

3.3.3 Mislabeling rate calculated for market form. To calculate the m. r. relative to the market form, 203 *bs* were further excluded from the count since the SPs analyzing these *bs* did not provide this information (Armani et al. 2015b; Armani et al. 2016). Thus, 3331 *bs* was considered and the overall *bs* number of each type of processing degree was modified accordingly (Table 4). The m. r. calculated for each market form is reported in Table 4. Overall, the m. r. appeared slightly higher in unprocessed *bs* (29.2%) respect to processed *bs* (27.2%), although this difference was not significative and both within the overall m. r. confidence interval (category B). Within unprocessed *bs,* m. r. in fresh *bs* (42.2%) is appeared significatively higher respect to frozen (22.5%) (p value <0.0001). Within processed *bs,* m. r. in highly processed seafood preparations (burger, minced, balls, cakes, filling, surimi, etc.) (49.0%) and salted *bs* (42.0%) are significatively higher respect to the other processed *bs* (p values <0.05).

3.3.4 Mislabeling rate calculated for distribution channel and geographical area. To calculate the m. r. relative to distribution channels, the 3534 *bs* mentioned in section 3.3.1 were used, and the overall *bs* number for each channel was modified accordingly. At retail level, the m. r. was 32.4% (category A2), with significant difference between large distribution (18.8% - category C1) and small distribution (54.2% - category A1) (p value <0.0001). The m. r. at mass caterers (15.0%) and official control activities (14.3%) were both found as significatively lower respect to retail level (p values <0.0001). To calculate the m. r. relative to the geographical area of collection, 2460*bs* were used. To the 2475 *bs* for which the geographical area was detailed (section 3.2.5), 15 *bs* where the taxonomic identity was not achieved were further removed. The m. r. was 43.2% in Southern Italy (category A2), that was found significatively higher respect to Central Italy (12.3% category C1), and Northern Italy (11.6% - category C1) (p values <0.0001). The m. r. observed for Islands was considered not significative as involving a total *bs* number <30.

The m. r. categories observed for publication year, species, market form, distribution channel and geographical area are summarized in Table 5.

4. Discussion

4.1 Years of publication, scientific journals and corresponding author/s affiliations

The distribution trend of publication throughout the years (2005-2022) was characterized by an increasing since 2015, which appeared to be in line with the global one. In fact, Luque & Donan (2019) observed that research on seafood fraud has especially grown with the advent of food forensics (e. g. DNA barcoding), with 51 papers published on the topic in 2015 compared to 4 in 2005.

Considering that EU was the territory with most publications on mislabeling (Luque & Donlan, 2019), we can suppose that the enactment of the Regulation (EU) No 1379/2013, which enhanced the application of DNA-testing to tackle falsely labeling practices, may have contributed to the SPs increasing. Note that, in their systematic review that was conducted up to December 2017, the inclusion criteria established by the authors led to the collection of 24 SPs in Italy (Luque & Donlan, 2019). In our study, a higher number of SPs (n=32) until December 2017 were included. To comment this, it is necessary to highlight that Luque & Donlan (2019) established specific inclusion criteria to select only papers that could contribute to the statistical estimation of global m. r. Among others, they especially excluded from the analysis cases where mislabeling was related to the strict interpretation of the expected trade name versus the trade name reported on the label. We suppose that this occurrence may be very common if studies from several countries are analyzed simultaneously, since expected trade names correspond to those reported to national official lists, may be extremely different from one country to another. This considered, the inclusion criteria identified in this review could be less stringent, with a higher number of recovered SPs and, consequently, a higher pool of data related to Italy, allowing to achieve a wider look of the national status of mislabeling. Furthermore, the higher number of SPs considered in this study might be recollected to the inclusion of studies originally not exclusively focusing on a mislabeling analysis but rather on the setting of DNA-testing tools for the further labeling check (section 3.2.3).

In this review we decided to exclude reports and articles on mislabeling that did not undergo a peer-reviewed process, that in some way can represent a quality control before publication, and we found that many SPs (32%) were published on five journals, namely "*Food Control*", "*Foods*", "*Food Research International*", "*Italian Journal of Food Safety*" and "*Journal of Agricultural and Food Chemistry*". In line with this, Luque & Donlan (2019) observed that 40% of the peer-reviewed publications selected in their systematic review were published in only five journals, including "*Food Control*", "*Food Research International*" and "*Journal of Agricultural and Food Chemistry*", confirming that these three journals are the major depositaries of scientific literature on this topic also at global level. Therefore, the inclusion of SPs published on international journals with high bibliometric indices (Impact Factor, SCImago Journal Rank, Source Normalized Impact per Paper) suggests the impact of studies and the interest of the scientific community on the topic. As regards the "*Italian Journal of Food Safety*", it is particularly required by Italian researchers as the official journal of the Italian Association of Veterinary Food Hygienists (AIVI) and as such it is suitable as a direct scientific information tool for continuous updating by the competent authority at national level.

Overall, research groups from the Departments of Veterinary Sciences were the most involved in the SPs publications, proving that veterinarians possess tools and skills to deal with this topic, especially respect to the knowledge of the legislation framework. Among them, Pisa (FishLab) and Bari Universities are at the top of the list, confirming that these two research units have specific competencies at national level.

4.2 Sampling: size, publication year, taxon, market form, distribution channel and geographical area.

A wide *bs* number range (from 3 to 290 *bs*) across the included SPs was observed. Accordingly, also in the systematic review by Luque & Donlan (2109), a highly variable sample size was observed (range: 8-4656; mean=194), as well as in other non-systematic reviews (Golden & Warner, 2014; Pardo et al., 2016; Warner et al., 2016). However, as detailed in the methodological section, only the *bs* collected on the Italian market and not belonging to reference specimens were considered in this study, so that the low *bs* number observed for some SPs may be due to this criterion. For instance, *bs* also collected both in Italy and in other countries were analysed in some SPs (Jerome et a., 2008; Giusti et al., 2019; Paracchini et al., 2019; Pardo et al., 2018). Respect to the overall *bs* number, a literature comparison was only possible by extrapolating data from the non-systematic review by Pardo et al. (2016), since the others did not provide the *bs* number for each country. About 350 samples were analysed in Italy from 2010 to 2015 (Pardo et al., 2016). In our study, over a double *bs* number (732 *bs*) were observed for the same period. The noticeable gap in numbers can be plausibly attributed to the fact that Pardo et al. (2016) did not conduct a systematic review, thus a comprehensive literature search was not required. No trend in the number of collected *bs* was observed across years, since the largest *bs* number was related with the largest SPs number.

In this study, data regarding taxon, market form, distribution channel were organized according to legislative provision and official reports (section 2.2) with the aim to define a standardized approach. Note that in the case of the market forms and distribution channels, such type of "official" categorization was not adopted by the available reviews on this topic, not even in the systematic review by Luque & Donlan (2019).

The analysis of the 219 species found in the included SPs highlighted a progressive evolution and expansion of the Ministerial list in response to the increased product demand and supply variety on the national market. This trend, in accordance with the requirement for a periodic updating of the list delegated to each Member State under Regulation (EU) 1379/2013 (Article 37) had already been described by Tinacci et al. (2019). In the study, specifically, the authors observed a continuous and significant updating of the designations included in each repealing Ministerial Decree till the list currently in application including over 1000 scientific names associated with more than 700 different official trade names. This aspect, among other causes, may be partially due to the contribute of scientific production investigating on seafood authentication and mislabeling assessment, that over the years have increasingly uncovered the presence of new species on the national market. The most iconic case is represented by the inclusion of two jellyfish species (*R. esculentum* and *R. pulmo*) in the Ministerial Decree n. 19105 of September the 22nd, 2017, presumably in consequence of the findings published by Armani et al. (2013).

As regard the taxon, fish, which resulted as the most representative in this study, is reported as the most sampled and analysed also at global level (Luque & Donlan, 2019; Pardo et al., 2016; Golden & Warner, 2014; Warner et al., 2016), while other seafood was less investigated. This aspect highlights a considerable gap for a comprehensive knowledge of the national market status in term of mislabeling occurrence, especially considering that other seafood categories are highly consumed in Italy. In fact, mussels, octopus and squids are included in the main commercial seafood in Italy

(EUMOFA, 2021). Factually, Kroetz et al. (2018) highlighted that several factors can be considered in the selection of products and species in mislabeling studies. The selection can be conducted in accordance with consumers demand trend but it is usually not planned in relation to national consumption; rather, products already identified from previous studies as being at a certain risk of mislabeling are the target. Hence, it can be inferred that the initial sampling plan can significantly contribute to a bias in the characterisation of the magnitude of the mislabelling rate. However, in this study the sampling of fish in term of MG/family/species and market form seems to be both influenced by the product commercial relevance (at EU and/or national level) and its attitude to be subject of fraudulent substitution practices. We found that unprocessed fillets or slices (fresh or frozen) were more representative respect to processed ones, mainly because in Italy the MG/families/species found as most investigated are especially marketed in this form. Also at global level, fillets and processed products have been reported as the most frequently sampled, with cods, especially Gadidae, found as the most investigated in term of *bs* number (Luque & Donlan, 2019). Within the last decade, cods have especially served as an exemplary case study for highlighting the impact of DNA technologies on the seafood authentication (Naaum, Warner, Mariani, Hanner, & Carolin,, 2016). This MG represents in fact the main group of exported species worldwide among fish (FAO, 2020a) and it accounts for more than one fifth of the apparent consumption of fishery and aquaculture products in EU, which is mainly supplied by imports (EUMOFA, 2021). Given its commercial value, *G. morhua* is reported among the most substituted species in the world (Feldmann, Ardura, Blanco-Fernandez, & Garcia-Vazquez, 2021; Naaum et al., 2016). This aspect is certainly encouraged by the fact it is mainly sold as frozen fillets worldwide (EUMOFA, 2020), as also observed in this study, where morphological key features of the whole specimens lack.

Similarly, the other market forms observed for cods *bs* in this study may drive fraudulent substitutions, such in the case of highly processed seafood preparations made of *G. chalcogrammus* and "baccalà" (processed salted) made of *G. morhua* or *G. macrocephalus. Gadus chalcogrammus* (Alaska pollock) is historically the main species used for surimi production worldwide but, due to its overexploitation, numerous previously underutilized fish species have started to be used posing this product to a high risk of species substitution (Galal-Khallaf, Osman, Carleos, Garcia-Vazquez, & Borrell,, 2016, Giusti et al. 2017; Keskin & Atar, 2012). Baccalà is instead one of the main heavy-salted products consumed in Mediterranean countries (Smaldone, Marrone, Palma, Sarnelli, & Anastasio, 2017). In Italy, it has been established that it can be obtained exclusively from *G. macrocephalus* (Pacific cod) and *G. morhua* (Atlantic cod) (Ministerial decrees of January 31st , 2008; Ministerial Decree n.19105 of September 22nd , 2017), but in other countries the legislation is different. For instance, the term "*Bacalao*" refers to all the species included in the genus Gadus in Spain, while "*Bacalada*" refers to *Micromesistius potessou*; in Portugal, also the species *Boreogadus saida* can be used for the "*Bachalau*" manufacturing; in Romania, only the species *Merlangius merlangus* is intended as "*Bacaliar*" (https://fish-commercial-names.ec.europa.eu/fish-names/home_en). Given this legislation discrepancy, cases of intentional or unintentional species substitution cannot be excluded, as also reported in literature (Di Pinto et al., 2013). The family Merlucciidae is also highly investigated worldwide in terms of mislabeling rates (Blanco-Fernandez et al., 2021; Luque & Donlan, 2019) and the species belonging to the genus *Merluccius* are of great interest due to their commercial relevance, especially in EU (Blanco-Fernandez et al., 2021). Currently, many of the *Merluccius* spp. have stocks under high fishing pressure and, since many species overlap their range of distribution with at least another congeneric species (FAO, 2020b). accidental mislabeling may occur due to the similar morphology of sympatric species, aggravating the fishing pressure on the threatened ones. In this respect, the market form observed in this study for *Merluccius* spp. (mainly processed breaded) may facilitate this event. The species *M. merluccius*, which is one of the main target species of the Mediterranean fisheries (Sioni et al., 2019), is instead mainly consumed in Italy as unprocessed fresh fillet (as confirmed by the observed *bs* sampling), with higher commercial value respect to the other *Merluccius* spp. However, annual catches have been halved from '90 to 2000-2013, indicating an overfishing status in several Mediterranean areas, with several scientists who highlighted the risk of a stock collapse (Russo et al., 2017). The reduced availability of this species, associated to its economic value, may encourage substitution practices with less valuable species, possibly deceiving consumers even respect to the purchasing of frozen-thawed instead of fresh products (Tinacci et al., 2018b).

Processed canned mackerels (family Scombridae) and herrings (Clupeidae) were also found as the most sampled *bs*. Mackerels are among the most commercially relevant fish group worldwide. The global market is primarily driven by the rising demand for canned tuna as consumers are shifting toward ready-to-eat products. EU consumption of tuna is largely supported by imports, consisting almost entirely of processed tuna, of which 30% is frozen and 70% includes prepared-preserved products (mainly canned). In fact, canned tuna is the most consumed seafood product also in EU (EUMOFA, 2021). Scombridae are among the families most investigated for m. r. also at global level (Luque & Donlan, 2019). Respect to Clupeidae, they represent the 18% of the EU traded small pelagic fish and processed products sold at retail level generally consist of whole, beheaded or filleted smoked exemplars, ready-to-eat, marinated or pickled, and canned delicacies, all of them also available on the Italian market. Semi-preserved anchovies (*E. encrasicolus*) are traditionally consumed within EU, with Spain and Italy among the major consumers, covering alone the 71% of the total EU consumption (EUMOFA, 2018). In Italy, anchovies are mainly consumed in form of ready-to-eat products, i. e. salted, marinated or in oil.

Other highly investigated species were mainly found as unprocessed (fresh or frozen) not whole, and they were *P. platessa* (MG flatfishes) and *X. gladius* (MG swordfish). Flatfishes are widely sampled for mislabeling evaluation also at global level (Luque & Donlan, 2019; Pardo et al., 2016). Italy is one of the main EU markets of *P. platessa,* and the supply is mainly based on imports, of which 95% of the volumes are fillets (mainly frozen) for consumption on the national market (EUMOFA, 2016). Also in the case of *X. gladius*, Italy is by far the main market in the EU (EUMOFA, 2018); according to the Institute for Agricultural and Food Market Services (ISMEA), it was the fifth most-consumed species in Italy in 2015, accounting for 3% and 5.5%, respectively, of volume and value of seafood household purchases (fresh or thawed slices). Also in these latter two cases, the market form (fillets and slices) highly poses the product at risk of fraudulent substitution.

Regarding the supply chain included in the studies, we found that sampling was mainly conducted at retail level, while *bs* from mass caterers and official control were together just above 20% of the overall *bs* number. Luque & Donlan (2019) reported that sampling was highly focused on restaurants and grocery stores, while wholesale venues, ports, and markets were less sampled. However, the different categorization proposed by the authors and the fact that retail level was fragmented in several sub-categories does not allow us to make a comparison between Italian and global level in term of distribution channels.

We especially believe necessary that surveys specifically focusing on seafood mislabeling in Italian mass caterers are provided, as for other EU countries (Christiansen et al., 2018; Pardo et al., 2018; Pardo & Jimenez, 2020), also considering that a great part of *bs* with no scientific name were found as collected therein. In fact, EU restaurants and other mass caterers are not obliged to put the mandatory information on their menus unless the Competent Authority requires so. They can do it voluntarily to improve the image and credibility of their business, as they are just obliged to keep such information and show the documents to the consumers if they require it (D'Amico, Armani, Gianfaldoni, & Guidi, 2016a). For this reason, fraudulent substitution may be more easily performed.

Also, it is opportune to underline the need to detail the sampling geographical area (missing for more than 30% of the *bs*), since this information may allow to better understand the mislabeling status across the entire national territory. In fact, the more extensive sampling observed in Apulia and Tuscany is essentially linked to the higher number of SPs performed in these regions, while for half of the Italian regions no data on mislabeling are currently available.

4.3 Mislabeling rates: publication year, taxon, market form, distribution channel and geographical area.

4.3.1. M. r. calculated overall and for year of publication. In this review, we decide to normalize the overall m. r. to the sample size, meaning that SPs with a greater number of samples were given a higher weight. This approach was also used by Oceana, the international organization dedicated to protecting and restore the oceans on a global scale, to calculate seafood mislabeling rates at global level through literature reviews (Golden & Warner, 2014; Warner et al., 2016). The latest one, examining global data on mislabeling until 2015, reported a global m. r. normalized to sample size of 19% (Warner et al., 2016). The data was not reported for countries, so that a comparison with m. r. in Italy cannot be performed. However, since the report also considered popular media sources, and public documents from governments and NGOs besides peer-reviewed journal articles, and not only those assessing the m. r. by molecular tools, we think that this comparison might not have been pertinent. Luque & Donlan (2019), who used a Bayesian meta-analyses approach, found a global m. r. of 24%, with a 95% highest density interval (HDI) from 20% to 29%. In Italy, they found a m. r of 26%, with 95% HDI from 18% to 34% (Luque & Donlan, 2019). The m. r. observed in our review (28.2% with a 95% CI from 26% to 30%) falls within the HDI reported by Luque & Donlan (2019), despite the different approaches used to calculate it. To remark that, also according to the diverse inclusion criteria that were adopted, an accurate m. r. comparison cannot be made. We also do not considered data from reviews reporting naïve m. r. since, in line with the observation by Luque & Donlan (2019), we think that they have limited utility for characterizing mislabeling.

Respect to the m. r per years, the lower values observed since 2016 might suggest that the increasing use of molecular tools to detect seafood frauds since 2015 has mitigated the mislabeling occurrence (section 4.2). However, it is appropriate to underline that the type of sampling across the years was essentially random; thus, considering that this aspect (especially the information related to species and market form) largely influences the m. r. for each year, a proper cause-effect link cannot be established. For instance, the higher m. r. observed in 2020 is probably related to the fact that, of the 111 *bs* found as mislabeled in that year, more than half (n=59; 53.2%) belonged to species showing m. r. within A category, namely *S. acanthias/S. blainville*, *M. mustelus/M. asterias/M. punctulatus* and *L. vulgaris*.

4.3.2 Mislabeling rate calculated for taxon. In the systematic review by Luque and Donlan (2019), species belonging to Serranidae and Lutjanidae had the highest estimated m. r. We found completely different outcomes, essentially related the characteristics of the Italian market, where most of these species are less consumed or not consumed at all. We found that the cases with highest m. r. (category A) can be typically considered as commercial frauds, since the substituent species generally have lower market price respect to the declared ones. It should be specified that the high m. r., discussed below cannot characterize alone the magnitude of the problem. For example, an extremely popular product with a low rate of mislabeling could yield a larger total quantity of mislabeled product than a frequently mislabeled product with limited consumer demand (Kroetz et al. 2018). In this respect, the higher m. r. was observed in non-conventional seafood (limited consumer demand) purchased within ethnic shops namely jellyfish-based products (Armani et al., 2013). The products, mainly labelled as the valuable *R. esculentum* were mainly substituted with *N. nomurai*, a species that has spread in the Chinese sea and that is reported to have an undesirable taste, which made it cheap and unpopular (Dong, Liu, & Keesing 2010). To consider that the products were sometimes marketed with a trade name referring to vegetables, highlighting that the labeling of the ethnic products often presents incongruences and deficits, as also reported in Armani et al. (2015b) and Armani et al. (2012b). The analyses on jellyfish-based products were performed with the aim to investigate a novel food marketed within the national territory (D'Amico, Leone, Giusti, Armani, 2016b).

Products labeled as *S. acanthias*/*S. blainville* ("*spinarolo*"), species found on Italian coasts and with prized meats, were found as often replaced with the cheaper *P. glauca*, (Filonzi, Chiesa, Vaghi, & Nonnis Marzano,2010; Marchetti et al. 2020). This can also be attributed to the decrease of *S. acanthias* in the Mediterranean Sea, now classified as vulnerable in the IUCN Red List (Table SM5). For this reason, the EU has recently prohibited the fishing, storage on board, trans-shipment and landing of this species (Council Regulation EU No 124/2019). Cases of replacement with *P. glauca* or other cheap shark species (e. g. *S. canicula, I. oxyrhincus*) were also observed for other valuable shark products, such as *M. mustelus/M. asterias/M. punctulatus* ("*palombo*") (Barbuto et al., 2010;

Marchetti et al., 2020). In contrast with how mentioned before, *bs* declared as "*palombo*" were especially substituted with *S. acanthias* in the SP by Barbuto et al. (2010), since the market price of this species were lower more than a decade ago and no fishing prohibition were imposed by EU legislation.

Perca fluviatilis (European perch) a freshwater species of high commercial interest living in the northern Italian rivers was found as often substituted with cheaper species that are farmed in highly polluted waters in the river Mekong in Asia and in African countries, represented by *P. hypophthalmus* (Striped catfish), and *L. niloticus* (Nile perch), respectively. Similar substitution patterns were observed for *E. marginatus* (Dusky grouper), another expensive and appreciated fish species. Factually, striped catfish was identified as the most substituted fish worldwide, and it is frequently disguised as wild, higher-value fish (Luque & Donlan, 2019; Warner et al. 2016). In the case of *Scomber scombrus*, it was hypothesized that the high m. r. is due to the fact that products labels often reported generic umbrella terms which can be ambiguously interpreted (Mottola et al., 2022).

The mislabeling cases of species belonging to cods generally concern the replacements with species included in the same family or order of the declared species. For instance, the highly relevant commercial species *G. morhua* and *G. macrocephalus*, the only species for which the name "baccalà" can be used in Italy (Ministerial Decrees n.19105 of September 22nd , 2017), were substituted with other less valuable species from the Gadidae and Lotidae families. Also, *M. merluccius* (European hake) was found as substituted with other species from Merluccidae or Gadidae. Similar incidents of replacement of *Gadu*s spp. or *Merluccius* spp. with congeners or species belonging to the family Gadidae or Merluccidae, have also been periodically described (Blanco-Fernandez et al., 2021; Grarcia-Vazquez et al., 2011; Munoz-Colmenero et al., 2015; Tinacci et al., 2018b, Helgoe, Oswald, & Quattro, 2020).

We observed high m. r. also for *X. gladius* (swordfish), which in Luke & Donlan (2019) showed instead a m. r. (4%) lower than the global m. r. This may be because this product is commercially relevant at national level. To underline that m. r. referring to cods and jacks were obtained from data collected during 14 years and 10 years, respectively, out of the 17-years considered period. Therefore, they can be considered more representative of the market situation respect to data arising from sporadic studies.

4.3.3 Mislabeling rate calculated for market form. We found no significative differences between m. r. in unprocessed and process *bs*. Contrariwise, significative differences were found within each category: within unprocessed significatively higher m. r. were observed in fresh (fillets and/or slices), and within processed significatively higher m. r. were observed in highly processed seafood preparations and processed salted. Luque & Donlan (2019), reported no statistical evidence that overall m. r. differs across product form at the global level. However, as the authors classified the market forms differently from our study, a proper comparison cannot be made.

Although the objective of mislabeling is mainly financial gain, the introduction of any substituted species into the food chain may result in health implication for consumers. Health risks associated with the consumption of mislabeled seafood may be defined based on the perspective on freshness, seafood allergies, contaminants such as mercury and other heavy metals, toxins including gempylotoxin, tetrodotoxin, ciguatoxin, and even the unintentional consumption of zoonotic parasites (Kusche & Hanel, 2021; Triantafyllidis et al., 2010; Williams et al., 2020). We are incline to believe that the real extent of health implication of the *bs* found to be mislabeled in the included SPs could be underestimated, since other types of hazards could have been involved. For example, Kusche & Hanel (2021) observed that the occurrence of ciguatera-prone species in the cohort of DNA-identified substituted fish was dramatically higher compared to the correctly labeled and the import tropical fishes especially poses an underestimated health risk for seafood consumers in Europe (Kusche & Hanel, 2021). This is equally true for zoonotic seafood borne parasites, since the substitution with species that are susceptible to specific parasites poses a clear human health (Williams, Hernandez-Jover, & Shamsi, 2020). In this respect, if we analyze *P. hypophthalmus,* one of the species found as substitute in this review and generally highly involved in substitution practices, it should be remark that, as farmed freshwater fish, it is vulnerable to infection by zoonotic parasites generally not associated with saltwater fish species. In particular, it has been identified infected with the fish born zoonotic trematodes *Centrocestus formosanus*, *Haplorchis taichui*, *H. pumilio* and *Chlonorchis sinensis* (Williams et al., 2020). The risk for consumers is particularly high if this species is substituted with valuable white fish species consumed raw, such as in sushi and sashimi, since humans become infected after consuming raw or undercooked fish containing viable meta-cercariae (Hung, Madsen, & Fried, 2013).

Even less is known about seafood mislabeling ecological and societal impact (Kroetz et al., 2018). It is relevant to highlight the importance of knowing the m. r. and the most frequent substitute species because this practices also harms fisheries and fishermen, allowing the introduction of illegal catches or not declared ones into the food markets (Feldmann et al.,2021). In this review, out of the 19 species reported as "*vulnerable*", "*endangered*" or "*critically endangered*" in the IUCN Red List of Threatened species (section 3.3.2), 14 are found as factually threatened, while the other five are mainly farmed and it is assumed that the mislabeling cases do not involved the wild threatened counterpart. Of these 14, shark species (*Galeorhinus galeus, Isurus oxyrhincus, M. mustelus, M. punctulatus Oxynotus centrina, Carcharodon carcharias, Carcharhinus brachyurus, Squalus brevirostris, S. acanthias, Alopias superciliosus*) were the most represented. In fact, according to the most recent systematic analysis performed by the International Union for the Conservation of Nature (IUCN) Shark Specialist Group (SSG), 74 of the 465 (15.9%) shark species included in the IUCN Red List are threatened (Dulvy et al., 2014). The presence of shark species that are threatened or are subject to global commerce regulation were also observed in mislabeled shark products collected in China (Zhang et al., 2021).

4.3.4 Mislabeling rate calculated for distribution channel and geographical area. In our study, m. r. was fund as significantly higher at retail level respect to mass caterers and official control levels. Contrary from our outcomes, global data analysed by Luque & Donlan (2019) showed no evidence for differences in mislabeling rates along the supply chain, although the m. r. observed for restaurants was higher. The higher incidence of mislabeling cases at mass caterers is instead reported in other reviews conducted in other EU countries (Christiansen, Fournier, Hellemans, & Volckaert, 2018; Pardo et al., 2018; Pardo & Jimenez, 2020). In our study, the m. r. observed at retail level are highly influenced by the contribution of the m. r. from small distribution (especially represented by ethnic retailers), since the large distribution alone, showed m. r. like that of mass caterers and official control activities. Ethnic activities are included in the small distribution, and despite a very good business organization, they are often characterized by deficiencies in traceability and labeling systems (Armani et al., 2013; Armani et al. 2015b; D'Amico et al., 2014). For instance, besides the case of jellyfish-based products labeled as vegetables (Armani et al., 2013), one *bs* collected at ethnic shop retail in 2015 (Armani et al., 2015b) was even labeled as *Carcharocles megalodon* (“Megalodon”), which is a shark that went extinct around 2.6 million years ago (Pimiento & Clements, 2014). To confirm this, in a study targeting ethnic food stores in UK to examine accuracy of traceability information available to consumers it was observed that about 41% of the samples were mislabeled, with a diverse range of poorly-known fish species, often sold without any label or with erroneous information (Di Muri et al., 2018). Differently from our outcomes, Pardo et al. (2016) observed that mislabeling incidents in mass caterers are significatively higher than retailers. However, only 10% of analyzed samples were obtained from mass caterers so that the authors underlined that specific studies should be conducted to confirm it (Pardo et al., 2016).

The low m. r. found for samples collected within official control are in line with overall m. r. reported in the aforesaid control plan performed by the EU Commission among Member State. In the aforesaid control plan performed an overall m. r. of 6%, but the rate at Member State level varied quite a lot, from 0 – 27 % (EU Commission Recommendation C(2015) 1558). Variations was related to many factors, e. g. which species of fish are more popular on their market, or the type of processing commonly used.

A statistical difference was observed for the first time among m. r. in Southern Italy respect to Central and Northern. In fact, it should be considered that the products found as highly mislabeled, such as *X. gladius* (swordfish), *P. fluviatilis, E. marginatus,* and baccalà, were especially sampled in this area.

4.4. Final remarks: strengths and weaknesses

4.4.1 Strengths. Considering the extent and severity of the food fraud impact on the economic, health and ecological aspects, the EU Commission and the Member States agreed on concrete measures and coordinated action to step up the fight against this practice. Besides strengthening the official control activities aimed at detecting frauds within all the EU territory, a consistent information exchange on food fraud notifications among Commission, Europol and the Competent Authorities designated by the Member States must be guaranteed within the RASFF portal. To facilitate information exchange and interpretation within this food fraud network, data standardization is essential. This aspect also drives the appropriate and successful outcomes of the risk assessment, the scientific process assumed as fundamental to establish the procedures for correct risk management at EU level. While for other issues involving the agri-food chain the data analysis is substantially defined and applied, for the food fraud field, and especially mislabeling practices, it still needs to be improved. In fact, current understanding of seafood mislabeling is largely limited to idiosyncratic studies without consistent methodologies or metrics (Kroetz et al., 2018; Luque & Donlan, 2019). It was in fact observed that all the available literature (systematic and non-systematic reviews, scientific papers, reports) categorized data differently, limiting the studies comparison and making those data scarcely usable for performing a target risk assessment. Although Italy was already investigated in previous surveys, detailed data divided for taxon, market form, distribution channel and geographical area were not provided or not easily extrapolated as provided aggregated with other data. Therefore, this is the first systematic review in which we tried to characterize seafood mislabeling at Italian level, trying to adopt a rigorous and standardized analytical approach that can be successfully used to assess mislabeling in other countries. Respect to the analysis of data related to sampling, we especially rigorously categorized the *bs* according to their taxon, market form, distribution channel and geographical area, to guarantee the synthesis, interpretation and reproducibility of information, as also recommended by EFSA (2010). In particular, dispositions provided by legislation (EU regulations and Italian Ministerial Decrees) and official reports were used for the categorization.

As regards the analysis of data related to mislabeling, we decided to calculate the overall m. r. weighted on the sampling size. The benefit of using a weighted average is that it allows the final average number to reflect the relative importance of each number that is being averaged. The importance to use weighted mean values has been highlighted by EFSA for standardizing the analysis of other parameters, such as the abundance of microplastics in food (EFSA, 2016), but no particular advices was provided for the m. r. calculation. and, as also observed in this study, m. r. are differently calculated in literature, so that outcomes from single studies are poorly informative for mislabeling characterization. We also decided to to fix 30 as minimum *bs* number to consider significative a mislabeling value

4.4.2. Weakness. Some bias of the included SPs inevitably affected the results of this review, and may consequently mislead the interpretation of the national mislabeling situation:

i) There is a notable lack of adequate sampling plans involving prior statistical analysis. First, sampling plan should include the prior statistical calculation of the "sample size" of the population under consideration, together with the "confidence interval" and "confidence level" selected in each specific study (Pardo et al., 2016). Overall, qualitative research has come under criticism for its lack of rigor in terms of there being little or no justifications given for the sample sizes that are actually used in research (Marshall, Cardon, Poddar, & Fontenot, 2013). Convenience sampling represent one of the most popular sampling techniques because it aligns the best across nearly all qualitative research designs. However, the sampling strategy reported in most of the included SPs did not consider a convenience, non-probabilistic sampling, structured to include a proportional number of products per type and brand. With a view to collect data that can be useful to evaluate mislabeling, we consider that it would be more suitable to exclude mixed sampling having low sample number for category and to opt for the analysis of a "representative" number of single product types (e. g. single MG/family/species, or market form, or distribution channel, or geographical area, etc.). In general, we think that specific and exhaustive guidelines on sampling strategy should be fixed, also by legislation, for investigating mislabeling, as for other typologies of control on food.

ii) There are not enough SPs aimed at assessing mislabeling in important seafood categories, namely taxa different from fish, and especially molluscs and crustaceans, which cover a substantial market share at national level. Therefore, is essential that the scientific community take action to investigate this taxon. In fact, data collected throughout most of the 17-years period only referred to commercially relevant fish MG. Also, data on processed products, especially those different from canned, are scarce. Considering their high predisposition to be mislabeled, complex multispecies seafood matrices should be more investigated, also considering that they cover a growing market segment.

ii) More efficient molecular techniques for species identification in the kind of products reported before should be set up and validated. For instance, the lack of data on seafood categories different from fish is probably because species identification by molecular tools is more challenging for some seafood, such as bivalve molluscs or crustaceans, since the DNA Barcoding approach relaying on the sequencing of a standard region of the Cytochrome Oxidase I (COI) gene (Hebert, Cywinska, Ball, & DeWaard, 2003), usually applied for most fish, is not effective (Armani et al., 2017; Tinacci et al., 2018b; Giusti et al., 2022). Additionally, the analysis of processed *bs*, especially in the case of complex multispecies matrices (e. g. surimi, burger, etc.), should be performed with the aid of more sophisticated authentication techniques based on metagenomic approaches and involving the use of Next Generation Sequencing Technologies (NGS). In fact, the standard molecular tools, such as DNA barcoding, are ineffective for species identification in these products (Franco, Ambrosio, Cepeda, & Anastasio , 2021; Haynes Haynes, Jimenez, Pardo, & Helyar, 2019). Despite of this, only 2 SPs included in this review applied NGS for the authentication of complex seafood products (Giusti et al., 2017; Piredda et al., 2022). However, this technique still presents high cost of analysis in term of reagents, equipment and expert personnel for the data analysis and therefore is still rarely used for research purposes related to food authentication. Since metagenomic approaches based on NGS technologies currently represent the most suitable technique for the analysis of complex matrices, it should become part of the routine activities of official and private laboratories operating in seafood authentication.

Conclusions

This systematic review and meta-analysis provided for the first- -time detailed information on seafood mislabeling on the Italian territory., according to seafood species, market form, distribution channels and geographical area. The inclusion of data only from peer-reviewed studies, the methodological rigor in the data extrapolation, and categorization based on dispositions provided by legislation (EU regulations and Italian Ministerial Decrees) and official reports represents an efficient analytical approach to support the obtained results. Therefore, outcomes from this study, could by providinge a risk assessment, and could serve for better implementing a risk management plan. In particular, driving more targeted official control activities, it can allow sing the definition of specific criteria to be considered in terms of seafood species, market form, distribution channels and geographical area. .In this respect, data may facilitate the identification of high-risk products, permitting to drive more targeted official control activities and undertake timely food inspections. . We think in fact that, the inclusion of data only from peer-reviewed studies, the methodological rigor in the data extrapolation, and categorization based on dispositions provided by legislation (EU regulations and Italian Ministerial Decrees) and official reports represents an efficient analytical approach to support the obtained the results. This is especially important considering the recent measures adopted by the EU Commission to fight against fraudulent practices.

Figure captions

Figure 1: Number of market blind samples (*bs*) analysed for each year of publication.

Figure 2. Number of blind samples (*bs*) belonging to the five most investigated generic market groups (MG) (mackerels, cods, herrings, flatfishes and jacks) through the years.

Figure 3. Number of market blind samples (*bs*) collected from the Italian market associated to each generic market group - MG (a), family (b) and species (c). MGs and relative families and species

have the same colour. Data in this figure only refer to MGs, families and species for which the *bs* number covers percentages ≥0.5% within each taxonomic level.

Figure 4. Details on unprocessed (a) and processed (b) *bs.* Both percentages and *bs* number are reported. *The SPs did not specify if they were fresh or frozen. The percentages were calculated on the overall number of a) unprocessed *bs* (n=2197), and b) processed *bs* (n=1379).

Figure 5. Mislabeling rates (m. r.) calculated for species (or higher taxonomic levels) with ≥30 *bs.* Number of mislabeled and not mislabeled *bs* for each species is reported. The different m. r. categories (A1, A2, B, C1, C2) are indicated in the right side.

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