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Recommended Citation

Liou, P., Banda, A., Isaacs, R.B., Hellberg, R.S., 2020. Labeling compliance and species authentication of fish fillets sold at grocery stores in Southern California. *Food Control* 112, 107137. https://doi.org/10.1016/j.foodcont.2020.107137

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Labeling Compliance and Species Authentication of Fish Fillets Sold at Grocery Stores in Southern California

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Title: Labeling compliance and species authentication of fish fillets sold at grocery stores in Southern California

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Abstract

Seafood mislabeling has numerous consequences, including economic deception and food safety risks. The focus of this study was to investigate fish species labeling, use of acceptable market names, and Country of Origin Labeling (COOL) compliance for fresh fish fillets sold at grocery store seafood counters in Southern California. A total of 120 fillets representing 16 different categories of fish were collected from 30 Perishable Agricultural Commodities Act (PACA)-listed grocery stores. Each sample underwent DNA barcoding to identify the species. Acceptable market names were confirmed using the FDA *Seafood List*. Samples were determined to be compliant with COOL if both the country of origin and the production method were declared in accordance with regulatory requirements. Species substitution was detected in 16 of the 120 samples (13.3%) and unacceptable market names were observed for an additional 11 samples (9.2%). The highest rates of species substitution were recorded for snapper (3/3), yellowtail (2/4), halibut (4/10), cod (3/10), and bass (2/7). COOL noncompliance was observed for 28 samples (23.3%): the country of origin was missing for 15 samples, production method was missing for 9 samples, and 4 samples were missing both. When all forms of mislabeling were considered, 47 of the 120 samples (39.2%) had at least one labeling error. The majority of grocery stores (25/30) had one or more samples with a mislabeling error. This study revealed species mislabeling as a continuous concern in the seafood industry, especially with higher-valued species. Furthermore, the lack of COOL compliance among retailers is concerning and suggests a need for increased focus on these regulations.

Keywords: acceptable market name, country-of-origin labeling, mislabeling, seafood fraud, species identification

1. Introduction

Seafood is a valuable protein source worldwide, with global per capita seafood consumption at over 20 kg per year (FAO, 2018). In the U.S., an estimated 7.3 kg of fish and shellfish were consumed per person in 2017, an increase of 0.5 kg from the previous year (NOAA, 2015). The top commercial fish consumed in the U.S. are salmon, tuna, tilapia, pollock, Pangasius, cod, and catfish (Delaware SeaGrant, 2018). Many fish fillets are similar in appearance yet have different market values, leading to the potential for species to be substituted for the purpose of economic gain (Hellberg & Morrissey, 2011). In addition to economic deception, species mislabeling can lead to health hazards, such as exposure to toxins like gempylotoxin and tetrodotoxin (Unicomb, Kirk, Yohannes, Dalton, & Halliday, 2002; Yancy et al., 2008). Mislabeling can also interfere with religious practices when kosher fish are substituted with non-kosher fish, and undermine the effectiveness of certification programs focused on reducing consumer demand for unsustainable fisheries (Willette et al. 2017).

In the U.S., intentional mislabeling of food is prohibited under 21 U.S.C. 343: Misbranded food. In order to avoid misleading consumers, the U.S. Food and Drug Administration (FDA) recommends that fish should be labeled using an acceptable market name provided in *The Seafood List*; however, numerous studies have reported seafood species substitution and mislabeling on the U.S. marketplace (Bosko, Foley, & Hellberg, 2018; Cline, 2012; FDA, 2018a; Khaksar et al., 2015; Mitchell & Hellberg, 2016; Shokralla, Hellberg, Handy, King, & Hajibabaei, 2015; Wang & Hsieh, 2016; Warner, Timme, Lowell, & Hirshfield, 2013; Willette et al., 2017; Wong & Hanner, 2008). A series of market surveys conducted across the U.S. revealed 18% species mislabeling from 731 fish collected from grocery stores, with snapper and grouper having the highest rates of mislabeling (Warner et al., 2013). Within California, studies have reported mislabeling rates of 2.2% (San Francisco) to 42% (Los Angeles) for fish samples collected at grocery stores (Bosko et al., 2018; Khaksar et al., 2015; Warner, Timme, Lowell, & Hirshfield, 2012; Willette et al., 2017). Some of the most commonly mislabeled fish detected in these studies were advertised as red snapper, yellowtail, yellowfin tuna, and salmon.

DNA-based methods are widely used for fish species authentication due to their accuracy and increased accessibility (Naaum & Hanner, 2016). DNA barcoding is a sequencing-based method that is commonly used for fish species identification (Naaum & Hanner, 2016). This method is based on genetic variation within a standardized region, which in animals is typically a ~650 base-pair (bp) fragment of the gene coding for cytochrome *c* oxidase subunit I (COI) (Hebert, Ratnasingham, & deWaard, 2003). COI generally exhibits high variability between species and conservation within species (Stern, Castro Nallar, Rathod, & Crandall, 2017). DNA barcoding has been adopted by the U.S. FDA for regulatory identification of fish species (Handy et al., 2011), and been successfully used to identify fish species in numerous studies (reviewed in Hellberg, Pollack, & Hanner, 2016). DNA barcode data for fish species is available through Fish-Barcode of Life (Fish-BOL), a global initiative to assemble a standardized reference sequence library for all fish species, and FDA's Regulatory Fish Encyclopedia (BOLDSystems, 2019; FDA, 2018b).

In addition to accurate species labeling, certain fresh and frozen seafood products (described below) must also follow Country of Origin labeling (COOL) regulations (Country of Origin Labeling for Fish and Shellfish, 7 C.F.R. § 60, 2009). COOL is a labeling law that requires retailers under the Perishable Agriculture Commodities Act (PACA) to provide consumers with information on the geographic origin and production method for fresh and frozen fish fillets, steaks, and nuggets that have not undergone transformation or further processing (USDA, 2017a, 2017b). The information must be legible and displayed in a conspicuous location, such as on a placard sign, label, sticker, band, or twist tie. Abbreviations for countries are not acceptable unless the codes cannot be mistaken for any other country or are common (USDA, 2017b). Furthermore, COOL regulations prohibit phrases such as “or,” “may contain,” and “and/or” to prevent confusion to consumers (USDA, 2017b). In addition to these regulations, foreign articles imported into the United States must be labeled with the correct country of origin according to 19 C.F.R. § 134.11, unless exempt by law.

About 90% of the seafood consumed in the U.S. is imported (NOAA, 2017); however, only a couple of peer-reviewed studies have investigated COOL compliance among retailers. One study conducted in Baltimore, MD, reported that 3.8% of the 628 fresh/frozen seafood products examined from 14 stores were not COOL compliant (Lagasse, Love, & Smith, 2014). Among the products, 1.1% did not state a country of origin and 2.7% did not state a procurement method (Lagasse et al., 2014). Another study surveyed catfish samples in Southern California and reported that 59% of the 32 catfish products collected from 31 grocery stores were not compliant with COOL regulations (Bosko et al., 2018). Among the 32 samples, 50% had incomplete or absent production method information and 31% were non-compliant for country-of-origin information. The higher levels of non-compliance observed by Bosko et al. (2018) may have been due to a number of factors, including differences in the number of retail locations visited, the fish types targeted, and the geographic locations for each study.

While numerous studies have been carried out on fish species substitution in the commercial marketplace, there is a lack of research that considers additional types of fish mislabeling. Therefore, the objective of this study was to examine fish fillets sold in Southern

California grocery stores for species authentication, use of acceptable market names, and COOL compliance.

2. Materials and Methods

2.1 Sample collection

A total of 120 fresh or thawed (previously frozen) fish fillets were collected from 30 grocery stores in Orange County, CA. Sixteen categories of fish were targeted based on their availability at grocery stores: bass, catfish, cod, halibut, mahi-mahi, Pangasius, rockfish, rockfish/snapper, salmon, snapper, sole, swordfish, tilapia, trout, tuna and yellowtail. The "rockfish/snapper" category included samples that were advertised as both snapper and rockfish. A maximum of 10 fish fillets were purchased per category with no more than two fish fillets from the same category purchased from the same retailer. All fish purchased for the study were from grocery stores licensed under PACA according to USDA's PACA Search Engine (https://apps.ams.usda.gov/pacasearch/). COOL information, species labeling, and price were photographed at the time of purchase (e.g., on placards, stickers, signs, labels, etc.) with the exact wording recorded. Figure 1 displays examples of COOL compliant labels collected in the study. Pictures were taken of the sign of the fish being sold, location of the COOL information, front/back of the packaged fish, receipts, and the unpackaged fish fillet. COOL compliance was assessed by examining the packaging of each product as well as any relevant information provided at the point of sale. In cases where the COOL information provided was questionable or unclear, an email was sent to COOL@ams.usda.gov per the USDA website (https://www.ams.usda.gov/rules-regulations/cool/questions-answers-consumers) to determine whether the product was considered compliant. Following collection, fish samples were transported to the laboratory in a cooler with ice packs and stored at 4°C. All fish were processed within 24 h of arrival to the laboratory. A subsample of the interior of the fish (~10 mg) was aseptically removed and placed in a sterile 1.5 mL microcentrifuge tube for immediate DNA extraction. The remaining sample was preserved at -80°C.

2.2 DNA extraction and quantification

DNA extraction was performed on each sample using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany), Spin-Column protocol with modifications described in Handy et al. (2011). Lysis was carried out at 56°C with shaking at 300 rpm in an Eppendorf ThermoMixer C (Hamburg, Germany) for 2 h. DNA was eluted in 100 µL of preheated AE buffer (37°C). The concentration of each DNA extract was measured using a Biophotometer Plus (Eppendorf). Any sample with a concentration >30 ng/µL was diluted with AE buffer to achieve a concentration ≤30 ng/µL, as described in Moore et al. (2012). Extracted DNA was stored at 4°C until use in PCR. Each set of DNA extractions also included a negative control in the form of a reagent blank without fish tissue.

2.3 PCR and DNA sequencing

All samples underwent full barcoding (655 bp) of the COI gene as described in Moore et al. (2012), except that the reaction volumes were doubled in order to improve workflow. Each reaction tube contained 12.5 µL 10% trehalose, 8.0 µL molecular grade H_2O, 0.5 OmniMix® HS Lyophilized PCR Master Mix bead (Cepheid, Sunnyvale, CA), 0.25 µL of each 10 µM COI full barcode primer (Table 1), and 2.0 µL of DNA template (≤30 ng/µL). Cycling conditions for full barcoding were 94ºC for 2 min; followed by 35 cycles of 94 ºC for 30 s, 55 ºC for 40 s, and 72 ºC for 1 min; with a final extension of 72 ºC for 10 min. All thermal cycling reactions were carried out using an Eppendorf Mastercycler nexus gradient.

Samples that could not be identified after the first round of DNA barcoding underwent repeat PCR using the full barcoding conditions described above, as well as mini barcoding using the Mini_SH-E primer set described in Shokralla et al. (2015). For mini-barcoding, each reaction tube contained 22.0 µL molecular grade H_2O, 0.5 OmniMix® HS Lyophilized PCR Master Mix bead, 0.50 µL of each 10 µM COI mini-barcode SH-E primer (Table 1), and 2.0 µL of DNA template. Cycling conditions were 95°C for 5 min; followed by 35 cycles of 94 °C for 40 s, 46 °C for 1 min, and 72 °C for 30 s; with a final extension of 72 °C for 5 min. In order to differentiate closely related tuna species, all tuna samples were also tested using a mini-barcode primer set targeting the control region (CR), as described in Mitchell and Hellberg (2016). Each reaction tube contained 20.5 µL molecular grade H_2O, 0.5 OmniMix® HS Lyophilized PCR Master Mix bead, 0.50 µL of each 10 µM CR mini-barcode primer (Table 1), and 3.0 µL of DNA template. Cycling conditions were 94°C for 2 min; followed by 35 cycles of 94 °C for 30 s, 49 °C for 40 s, and 72 °C for 1 min; with a final extension of 72 °C for 10 min.

PCR products were confirmed using pre-cast 2% agarose E-Gels (Invitrogen, Carlsbad, CA) run for 15 min on an E-Gel iBase (Invitrogen). Each well was loaded with 4 µL PCR product and 16 µL sterile deionized water. Image results were captured using FOTO/Analyst Express (Fotodyne, Hartland, WI) and Transilluminator FBDLT-88 (Fisher Scientific, Waltham, MA) and visualized with FOTO/Analyst PCImage (version 5.0.0.0, FOTODYNE). PCR products were purified using ExoSAP-IT (Affymetrix, Santa Clara, CA) according to the manufacturer’s instructions. Next, the samples were sequenced bidirectionally with M13 primers at the GenScript facility (Piscataway, NJ). Sequencing was carried out using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) and a 3730xl Genetic Analyzer (Applied Biosystems).

2.4 DNA sequence analysis

Raw sequence data was assembled using Geneious R7 (Biomatters, Ltd., Auckland, New Zealand) and trimmed to the target regions for the 655 bp full-length COI barcode, 226 bp COI mini-barcode, or 236 bp CR mini-barcode. Full-length COI barcodes were considered successful if they passed the QC parameters described by Handy et al. (2011): bidirectional sequences with ≥ 500 bp and < 2% ambiguities or single reads with ≥ 500 bp and ≥ 98% high quality bases. COI and CR mini barcodes were considered successful if they passed the QC parameters utilized by Pollack et al. (2018): bidirectional sequences with ≥ 76% of the target length and < 2% ambiguities or single reads with ≥ 76% of the target length and ≥ 98% high quality bases. The full and mini-barcode COI sequences were queried against the Species Level Barcode Records in the Barcode of Life Database (BOLD) and CR mini-barcodes were queried against GenBank using the Basic Local Alignment Search Tool (BLAST). Common names and acceptable market names for each identified species were determined using *The Seafood List* (FDA, 2018a). For species not listed in *The Seafood List*, FishBase was used to determine the common names (FishBase, 2018).

3. Results and Discussion

3.1 DNA barcoding results

All of the 120 fish fillets collected were sequenced with at least one of the COI barcoding methods described above and all samples had at least one top species match in BOLD with >99% genetic similarity (Table 2). The majority of samples (n = 116) were sequenced using the COI full barcode primer set and the remaining four samples were sequenced with the COI mini-barcode primer set. The four samples that were only successful with mini-barcoding were identified as Atlantic salmon (*Salmo salar*; n = 2), Patagonian toothfish (*Dissostichus* *eleginoides*; n = 1), and Antarctic toothfish (*Dissostichus mawsoni*; n = 1). Among the 120 fillets tested, 82 were identified to the species level (i.e., showed a top match to a single species in BOLD) using COI full or mini-barcoding. This included all samples labeled as bass, catfish, salmon, snapper, sole, swordfish, trout, yellowtail and most samples labeled as cod, halibut, mahi-mahi, rockfish (Table 2).

Among the 38 samples that were not identified to the species level with COI full or mini-barcoding, 23 were identified to the genus level (i.e., showed a top match to multiple species from the same genus). These included the majority of the tilapia and tuna samples and a few samples of halibut, mahi-mahi, and rockfish (Table 2). Most of the tilapia samples had top matches to *Oreochromis* hybrids and therefore could not be identified at the species level. Many species of tuna are closely related and previous studies have also reported an inability to differentiate species based on COI DNA barcoding (Pollack et al., 2018; Shokralla et al., 2015). These samples underwent further analysis with the CR mini-barcodes to verify species. All 10 tuna samples were successfully sequenced using the CR mini-barcode primer set and identified as yellowfin tuna (*Thunnus albacares*; n = 5), Pacific bluefin tuna (*Thunnus orientalis*; n = 2), albacore tuna (*Thunnus alalunga*; n = 1), southern bluefin tuna (*Thunnus maccoyii*; n = 1), and *Thunnus* spp. (n = 1). The CR mini-barcodes showed 100% query coverage and 95-100% genetic similarity to the top species matches in GenBank, consistent with the results of Mitchell and Hellberg (2016).

Samples with top matches from multiple genera were primarily from the Pangasius (n = 9) and cod (n = 5) categories (Table 2). The Pangasius samples showed top matches to records from the genera *Pangasianodon* and *Pangasius*, which are both within the Pangasiidae family, while the cod samples showed equivalent matches to records from the genera *Gadus* and *Boreogadus*, which are both within the Gadidae family.

3.2 Species substitution

Species substitution was detected in 16 of the 120 fish fillets (13.3%) examined in this study (Table 3). Among the 16 fish categories tested, 7 had at least one sample with species substitution. The highest rate of substitution was observed for the snapper fillets (3/3), followed by yellowtail (2/4), halibut (4/10), cod (3/10), and bass (2/7). The Pangasius and tuna categories each had one sample with species substitution. Categories with no species substitution detected included: catfish, mahi-mahi, rockfish, rockfish/snapper, salmon, sole, swordfish, tilapia, and trout. Similar to the results of the current study, previous market surveys in the U.S. also found relatively high rates of mislabeling among snapper, halibut, and cod, and yellowtail products (Hu, Huang, Hanner, Levin, & Lu, 2018; Khaksar et al., 2015; Shehata, Naaum, Garduno, & Hanner, 2018; Warner et al., 2013; Willette et al., 2017). Of the 30 stores sampled in the current study, 13 had at least one incidence of species substitution. The three most expensive categories of fish had relatively high rates of species substitution: snapper, bass, and halibut were on average the highest-priced fish categories at US $99.93/kg, $88.18/kg, and $49.01/kg, respectively.

According to *The Seafood List*, the name "red snapper" is only acceptable for *Lutjanus campechanus* (FDA, 2018a). However, none of the fillets advertised as "red snapper" in this study were identified as *L. campechanus* (Tables 2-3). As shown in Table 3, the three substituted "red snapper" fillets were identified as blackspotted rockfish [(*Sebastes melanostictus*) (n = 1)] and madai [(*Pagrus major*) (n = 2)]. According to the California Code of Regulations (14 CCR §103), "Pacific red snapper" can be used as a common name for certain species of rockfish including widow rockfish (*Sebastes entomelas*) and vermilion rockfish (*Sebastes miniatus*). However, none of the samples collected in this study were specifically labeled as "Pacific red snapper." The two "red snapper" samples identified as madai were sold as "fresh red snapper" farmed in Japan ($132.28/kg) and "premium red snapper" wild caught in Japan ($154.32/kg) (Fig. 1a). Madai is a type of sea bream that is recognized as genuine snapper in sushi culture and this may have led to confusion over the acceptable market name (Hu et al., 2018). Consistent with the results of the current study, Khaksar et al. (2015) also reported 100% of "red snapper" samples to be mislabeled, with 8 of the 16 samples identified as madai and the other 8 identified as tilapia. Similarly, Warner et al. (2013) reported a high rate of red snapper mislabeling (113 of 120 samples), with samples identified as various species, including madai (n=5) and numerous types of rockfish (n=30). These results, along with those of other studies (Hsieh, Woodward, & Blanco, 1995; Hu et al., 2018; Marko et al., 2004; Shehata et al., 2018; Willette et al., 2017), indicate that red snapper substitution continues to be a major problem.

According to 21 CFR §102.57, the term "halibut" can only be associated with Atlantic halibut (*Hippoglossus hippoglossus*) or Pacific halibut (*Hippoglossus stenolepis*). However, four of the ten fillets in this study advertised as "halibut" or "Pacific halibut" were identified as California flounder (*Paralichthys californicus*) (Table 3). Interestingly, "California halibut" is listed as a vernacular name for California flounder on *The Seafood List* and it is the name used to refer to *P. californicus* in the California Fish and Game Code (e.g., §8391). However, as stated by the FDA, vernacular names are generally not acceptable market names and use of these names may lead to misbranding. Consistent with these results, Warner et al. (2013) also detected California flounder labeled as "Pacific halibut" in four samples purchased in Northern

California. Willette et al. (2017) found that 89% of marketed halibut was actually flounder (*Paralichthys* spp.), although none were identified as California flounder.

Among the cod samples, two were advertised as Pacific cod (*Gadus microcephalus*) but identified as Atlantic cod (*Gadus morhua*) and one was advertised as rock cod (*Lotella rhacina* or *Pseudophycis barbata*) but identified as redbanded rockfish (*Sebastes babcocki*) (Table 3). Mislabeling Atlantic cod as Pacific cod could undermine conservation efforts at the retail level, as Atlantic cod is considered vulnerable by the International Union for Conservation of Nature (IUCN) Red List (IUCN, 2019). According to NOAA Fisheries, Atlantic cod populations are below target levels; however, U.S. wild-caught Atlantic cod is being sustainably managed with limited harvesting and rebuilding plans in place (NOAA, 2019). Of note, one of the Atlantic cod samples (P031) listed the U.S. as the country of origin, while the other sample (P001) listed Iceland. Similar to the results of this study, Warner et al. (2013) reported a mislabeling rate of 28% for cod species, including Atlantic cod mislabeled as Pacific cod and redbanded rockfish mislabeled as rock cod, while Shehata et al. (2018) also found Atlantic cod mislabeled as Pacific cod.

The bass category included one fillet labeled as “seabass (Patagonian toothfish)” and six fillets labeled as “Chilean seabass.” As shown in Table 3, the sample labeled as “seabass (Patagonian toothfish)” was determined to be substituted because Patagonian toothfish (*Dissostichus eleginoides*) is a different species than Antarctic toothfish (*Dissostichus mawsoni*). Within the “Chilean seabass” samples, one was identified as swordfish (*Xiphias gladius*). The substitution of Chilean seabass with swordfish could have been intentionally carried out for economic gain, as the average price of swordfish in this study was US $28.55/kg compared to US $69.31/kg for samples labeled as Chilean seabass. The substitution is also a health concern as swordfish is not recommended for certain populations (i.e. pregnant women, young children) due to mercury levels, while Chilean sea bass is listed as a "good choice" (FDA, 2019).

The Pangasius, tuna, and yellowtail categories each had one sample found to be substituted (Table 3). Interestingly, a sample labeled as "swai" was identified as blue-spotted stingray (*Neotrygon kuhlii*). Economically motivated adulteration in this case seems unlikely, as the average price of the Pangasius samples in this study was relatively low (US $9.91/kg, range $8.79-13.21/kg). The substituted tuna sample was labeled as "yellowfin tuna" but identified as southern bluefin tuna. Southern bluefin tuna is considered critically endangered according to the IUCN Red List (Collette, Chang, et al., 2011), while yellowfin tuna is considered near threatened (Collette, Acero, et al., 2011). The country-of-origin information for this tuna sample was conflicting, with "Indonesia" listed on the placard and "Fiji" on the label. Economically motivated adulteration seems unlikely, as this sample was marketed at US $22.05/kg as compared to US $59.52 for the other yellowfin tuna sample in this study. Lastly, two samples (P035 and P104) advertised as "yellowtail" were identified as buri (*Seriola quinqueradiata*). Although buri shares the same genus as yellowtail (*Seriola lalandi*), they are two distinct species. In addition, the country of origin and production method were both missing for P035 (Fig. 2d) . Buri is a common substitute for yellowtail, as Warner et al. (2013) previously identified 24 out of 26 "yellowtail" samples as buri. The authors indicated that the deception was likely unintentional, as buri is often called "yellowtail" at sushi restaurants. Interestingly, the average cost of actual yellowtail samples in the current study was US $7.67/kg, while the average cost of the "yellowtail" samples identified as buri was much higher, at US $42.99/kg.

3.3 Acceptable market name

The use of an acceptable market name to identify seafood sold in interstate commerce is important in order to ensure proper labeling and avoid misleading consumers (FDA, 2018a). Among the 120 samples, 11 samples from 10 stores were mislabeled due to the use of an unacceptable market name (Table 4). When samples with species substitution and unacceptable market names were combined, the overall rate of mislabeling was 22.5% (27/120). The category with the greatest number of unacceptable market names was salmon (5/10), followed by rockfish/snapper (2/2), cod (2/10), and Pangasius (2/10). The two samples of rockfish/snapper were found to have unacceptable market names because of conflicting labeling information: one sample was labeled as "Fresh Pacific Snapper Filet" on the placard and "Pacific Rockfish Fillet Wild-Fresh" on the label, while the other was labeled as "Fresh Rockfish Red Snapper" on the placard and "Rock Fish Fillets" on the label. However, "Pacific snapper" is only acceptable for *Lutjanus peru* and, as previously mentioned, "red snapper" is only acceptable for *Lutjanus campechanus*. In the state of California, certain rockfish species may be labeled as "Pacific Red Snapper" according to the California Code of Regulations §103. However, this name was not used for any of the rockfish samples collected.

The five mislabeled salmon samples were labeled as "salmon" and identified as "Atlantic salmon." Although these fillets were labeled with the correct category of fish, none of them used the complete name of "Atlantic salmon" as specified by *The Seafood List*. Another mislabeling trend was the use of multiple names on the same product that refer to different species. For example, one of the mislabeled Pangasius samples was marketed as both "swai" and "basa" and another was marketed as "red fish basa." "Swai" and "basa" refer to two different species as do "red fish" and "basa." "Redfish" appears as a vernacular name for a number of species in *The Seafood List*, including sea bass, ocean perch, and sockeye salmon. In another case, a fillet identified as sablefish (*Anoplopoma fimbria*) was labeled with the vernacular name of "black cod." The other mislabeled cod sample was advertised as "lind cod." Lind cod is not listed in *The Seafood List* and it may be a possible misspelling of ling cod (*Molva movla*). However, the sample had equivalent species matches to Pacific cod (*Gadus macrocephalus*)/Arctic cod (*Boreogadus saida*)/Greenland cod (*Gadus ogac*), none of which are associated with an acceptable market name of "ling cod."

3.4 COOL compliance

To comply with COOL regulations, the country of origin and production method must be stated legibly in a conspicuous location at the point of sale. Examples of COOL-compliant samples collected in this study are shown in Figure 1. COOL noncompliance was observed for 28 of the 120 samples (23.3%) in this study (Table 5). A greater number of samples were not compliant in their country-of-origin statement (n = 15) compared to samples that were noncompliant for production method (n = 9). Four additional samples were noncompliant for both country of origin and production method information. Only four of the fish categories (i.e., cod, rockfish, rockfish/snapper, and trout) had samples that were 100% COOL compliant. Each of the remaining categories had at least one incidence of COOL noncompliance, with tuna having the highest number of non-compliant samples (n = 5). At least one sample from 15 of the 30 stores (50.0%) sampled had an incidence of COOL noncompliance.

Samples were considered not compliant in their country-of-origin statement for several reasons: ten samples were missing a country of origin or stated "Other" as the country of origin; six listed multiple countries; and three did not use a valid country name. The samples with multiple countries had contradictory information on the label as compared to the placard. For example, one sample was a "red snapper" fillet (P019) that listed Canada on the placard and

Brazil on the label. Of note, this sample was substituted with blackspotted rockfish and also contained contradictory production method information, declaring “Farm Raised” on the placard and “Wild” on the label. Another sample with contradictory information was a catfish fillet (P018) that declared “Product of China” on the placard and “Product of Ecuador” on the label. Interestingly, the label for this sample appeared have been intended for use with a shrimp product, as it read “26-30 Raw Headless Shri Previously Frozen Farmed.” One of the samples (P032) with an invalid country name stated “Product of Tahiti” instead of the country name of French Polynesia. The other two samples with invalid country names were bass fillets that listed “Korea” (P029) or “Korean” (P105) (Fig. 2a) as the country of origin. Because South Korea and North Korea are two separate countries, simply stating “Korea” is considered insufficient (K. Becker, personal communication, October 10, 2018). Of note, the sample that listed “Korea” as the country of origin was also found to be mislabeled on the basis of species: it was advertised as “seabass (Patagonian toothfish)” but identified as Antarctic toothfish.

Among the 13 samples that were noncompliant with regards to declaring the production method, ten samples did not state the production method, two had unclear wording, and one had contradictory information. The two samples with unclear wording were a mahi-mahi fillet with the declaration “Born, Raised, Harvested China” (Fig. 2b) and a tilapia fillet with the declaration “BRN,RAISD&HARVST CHINA.” These statements reflect the legal designations required for muscle cuts of meat from animals slaughtered in the U.S. (7 CFR §65.300 d) and they are not acceptable for conveying production method for fish and shellfish (K. Becker, personal communication, April 9, 2019).

Interestingly, two samples with COOL information listed a country of origin or production method that was not consistent with the labeled species. In one case, a sample labeled as “Wild Caught Pacific Cod” (P001) listed Iceland as the country of origin. While Pacific cod can be found in the waters off of western Greenland, its geographic range does not extend to Iceland (Luna & Capuli, 2019). The sample was identified to be Atlantic cod, which is a major fishery in Iceland (FAO, 2010). Another sample was labeled as farmed mahi-mahi (no country of origin stated); however, the Food and Agriculture Organization of the United Nations (FAO) does not have production statistics for farmed mahi-mahi (FAO, 2018).

The rate of COOL noncompliance in this study (23.3%) was mid-range compared to previous studies. Lagasse et al. (2014) found only 3.8% COOL noncompliance from the 628 seafood products examined in their study. However, their samples were collected from only eight retail outlets compared to 30 grocery stores in this study and included both fresh and frozen products. COOL compliance surveillance conducted by the Agricultural Marketing Service (AMS) in 2016 revealed 10% COOL noncompliance among 79,928 fish and shellfish products from over 3,000 retail store facilities across the United States (K. Becker, personal communication, June 21, 2017). On the other hand, Bosko et al. (2018) reported 59% COOL noncompliance among 32 fresh/frozen catfish samples collected from grocery stores. In comparison, the current study found a lower rate of noncompliance (33.3%) among the 10 catfish products analyzed. While relatively high rates of COOL noncompliance have been observed in studies specific to Southern California, these differences may be due to variation in sampling design rather than regional differences in COOL compliance. A more extensive study focused on comparing COOL compliance in multiple geographic regions should be carried out in order to investigate these differences further.

3.5 Overall mislabeling

When considering all forms of mislabeling investigated in this study (i.e., species substitution, unacceptable market name, and/or COOL noncompliance), 47 of the 120 samples (39.2%) had at least one labeling error. Eight samples exhibited COOL noncompliance combined with species mislabeling (i.e., species substitution or unacceptable market name). Among these samples, there were seven instances of species substitution and one use of an unacceptable market name. These samples were from a range of categories, including bass, halibut, Pangasius, salmon, snapper, tuna, and yellowtail. Among the 30 stores sampled, 24 stores (80.0%) had at least one incidence of species mislabeling or COOL noncompliance.

4. Conclusions

This study revealed species mislabeling and COOL noncompliance across various fish categories in grocery stores in Southern California. The results of the current study combined with previous research indicate that mislabeling of fish species continues to be a problem. Several instances of higher-value species substituted with species of lesser value were detected in this study, such as halibut substituted with California flounder. However, many instances of species mislabeling appeared to be a result of confusion in naming fish associated with sushi culture (e.g., use of the term "madai" for red snapper) or a misunderstanding of California state and federal labeling laws (e.g. use of "Pacific halibut" for California flounder), rather than carried out for economic gain. Numerous errors associated with COOL compliance were also observed, including lack of a country-of-origin statement, lack of production method, and confusing or contradictory wording. Non-compliant samples may be due to a lack of consistency at certain grocery stores, as some samples displayed contradictory information between the placard and the label and others used wording meant for cuts of meat instead of fish (e.g. "born, raised, & harvested"). Accurate and compliant labeling is an important aspect in determining appropriate food safety measures, promoting seafood conservation, and allowing consumers to make informed choices associated with seafood consumption. As a labeling law, COOL provides transparency in the supply chain to consumers. The high number of stores (80.0%) and fish products (39.2%) that had at least one mislabeling error indicates an area of concern and a need for further monitoring as well as greater enforcement of regulations.

Acknowledgements

This work was supported in part by a grant from the National Science Foundation, Division of Earth Sciences, NSF-EAR #1757991. Additional funding support was received from Chapman University, Schmid College of Science and Technology. We would like to thank Dr. Kenneth Becker from U.S. Department of Agriculture and Spring Randolph from U.S. Food and Drug Administration for their help in providing resources and clarification related to regulatory questions.

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Table 1. Primer sets used in this study

Primer set	Primer name	Primer direction	Primer sequence (3’-5’) [a]	Barcode length	Reference
COI full barcode	FISHCOILBC_ts	forward	CACGACGTTGTAAAACGACTCAACYAATCAYAAAGATATYGGCAC	655 bp	Handy et al. (2011); Moore et al. (2012)
	FISHCOILBC_ts	reverse	GGATAACAATTTCACACAGGACTTCYGGGTGRCCRAARAATCA		
COI mini-barcode (SH-E)	Mini_SH-E	forward	CACGACGTTGTAAAACGACACYAAICAYAAAGAYATIGGCAC	226 bp	Shokralla et al. (2015)
	Mini_SH-E	reverse	GGATAACAATTTCACACAGGCTTATRTTRTTTATICGIGGRAAIGC		
CR mini-barcode	Tuna CR_F	forward	CACGACGTTGTAAAACGACGCAYGTACATATATGTAAYTACACC	280 bp	Mitchell and Hellberg (2016)
	Tuna CR_R1	reverse	GGATAACAATTTCACACAGGCTGGTTGGTRGKCTCTTACTRCA		
	Tuna CR_R2	reverse	GGATAACAATTTCACACAGGCTGGATGGTAGGYTCTTACTGCG		

[a]underlined segment indicates M13 tails

Table 2. Combined results of full and mini-DNA barcoding for fish fillets tested in this study (n = 120). Values are displayed as the number count.

Category	Number of samples	Identified to species level	Identified to genus level	Identified to multi-genus level	Samples with species mislabeling[a]
Bass	7	7	--	--	2
Catfish	10	10	--	--	0
Cod	10	5	--	5 (*Gadus* and *Boreogadus*)	5
Halibut	10	8	2 (*Hippoglossus*)	--	4
Mahi-mahi	6	5	1 (*Coryphaena*)	--	0
Pangasius	10	1	--	9 (*Pangasianodon* and *Pangasius*)	3
Rockfish	6	5	1 (*Sebastes*)	--	0
Rockfish/snapper	2	1	1 (*Sebastes*)	--	2
Salmon	10	10	--	--	5
Snapper	3	3	--	--	3
Sole	10	10	--	--	0
Swordfish	10	10	--	--	0
Tilapia	10	--	9 (*Oreochromis*)	1 (*Oreochromis* and *Pseudocrenilabrus*)	0
Trout	2	2	--	--	0
Tuna	10	1	9 (*Thunnus*)[b]	--	1
Yellowtail	4	4	--	--	2
Total	120	82	23	15	27

[a]Refers to samples with species substitution or unacceptable market name
[b]Eight of these samples were identified to the species level with the CR mini-barcode

Table 3. Instances of species substitution detected in this study (n = 16)

Sample ID	Category	Product name on placard[a]	Product description on label [a]	Expected species	Price paid (US $/kg)	Identified species
P029	Bass	Seabass (Patagonian toothfish)	Seabass (Patagonian Tooth Fish)	Patagonian toothfish (*Dissostichus eleginoides*)	88.18	Antarctic toothfish (*Dissostichus mawsoni*)
P101	Bass	Seabass Chilean Portions Minimum 5 oz Previously Frozen	Seabass Chilean Portions Minimum 5 oz Previously Frozen	Antarctic toothfish (*Dissostichus mawsoni*) or Patagonian toothfish (*Dissostichus eleginoides*)	94.01	Swordfish (*Xiphias gladius*)
P001	Cod	Fresh Wild Caught Pacific Cod Fillets	True Cod Fillet Fresh	Pacific cod (*Gadus microcephalus*)	30.86	Atlantic cod (*Gadus morhua*)
P031	Cod	Pacific Cod	Pacific Cod Fillet	Pacific cod (*Gadus microcephalus*)	33.07	Atlantic cod (*Gadus morhua*)
P063	Cod	Rock Cod Fillet	Fillet of Rock Cod	Rock cod (*Lotella rhacina* or *Pseudophycis barbata*)	8.82	Redbanded rockfish (*Sebastes babcocki*)
P061	Halibut	Fresh Halibut Steak	Halibut Steak	Atlantic halibut (*Hippoglossus hippoglossus*) or Pacific halibut (*Hippoglossus stenolepis*)	15.42	California flounder (*Paralichthys californicus*)
P065	Halibut	Halibut Steak	Halibut Steak	Atlantic halibut (*Hippoglossus hippoglossus*) or Pacific halibut (*Hippoglossus stenolepis*)	15.43	California flounder (*Paralichthys californicus*)
P069	Halibut	Halibut Steak	Halibut Steak	Atlantic halibut (*Hippoglossus hippoglossus*) or Pacific halibut (*Hippoglossus stenolepis*)	24.25	California flounder (*Paralichthys californicus*)
P099	Halibut	Fresh Central Pacific Halibut Fillet	Fresh Central Pacific Halibut Fillet	Pacific halibut (*Hippoglossus stenolepis*)	61.73	California flounder (*Paralichthys californicus*)
P047	Pangasius	Frozen Red Swai Fillet	Frozen Red Swai Fillet	Sutchi catfish (*Pangasianodon hypophthalmus*)	8.82	Blue-spotted stingray (*Neotrygon kuhlii*)

P019	Snapper	Red Snapper Fillet	Whole Clean Red Snapper Fresh/Wild	Red snapper (*Lutjanus campechanus*)	13.19	Blackspotted rockfish (*Sebastes melanostictus*)
P117	Snapper	N/A (no placard)	Fresh Red Snapper Sashimi	Red snapper (*Lutjanus campechanus*)	132.28	Madai (*Pagrus major*)
P118	Snapper	N/A (no placard)	Premium Red Snapper	Red snapper (*Lutjanus campechanus*)	154.32	Madai (*Pagrus major*)
P074	Tuna	Yellowfin Ahi Tuna Steak Previously Frozen	Tuna Yellow Fin/Ahi Steak Skin-Off Previously Frozen - CO	Yellowfin tuna (*Thunnus albacares*)	22.05	Southern bluefin tuna (*Thunnus maccoyii*)
P035	Yellowtail	N/A (no placard)	Sushi Yellowtail	Yellowtail (*Seriola lalandi*)	55.12	Buri (*Seriola quinqueradiata*)
P104	Yellowtail	N/A (no placard)	Yellowtail Kirimi	Yellowtail (*Seriola lalandi*)	30.86	Buri *(Seriola quinqueradiata)*

[a]COOL information not included unless part of product name

Table 4. Samples found to have unacceptable market names (n = 11) according to the FDA *Seafood List*. Note: FDA recommends using the common name as the market name unless prohibited by regulation or law.

Sample ID	Category	Product name on placard	Product description on label	Identified species (common name and scientific name)	Acceptable market name(s) other than the common name	Comments
P085	Cod	N/A (no product name on placard)	Fresh Lind Cod	Pacific cod (*Gadus macrocephalus*)/ Arctic cod (*Boreogadus saida*)/ Greenland cod (*Gadus ogac*)[a]	Cod or Alaska cod (for Pacific cod)	Possible misspelling of "ling cod", a vernacular name for *Molva molva*
P103	Cod	N/A (no placard)	Black Cod Kirimi	Sablefish (*Anoplopoma fimbria*)	Sablefish	Black cod is a vernacular name for sablefish
P013	Pangasius	N/A (no placard)	Swai Basa Fillet	Sutchi catfish (*Pangasianodon hypophthalmus*)[b]/ *Pangasius bocourti*[c]/*Pangasius krempfi*[c]/*Pangasius djambal*[ac]	Swai or Sutchi or Striped Pangasius or Tra/Basa	Swai and Basa refer to two separate species
P039	Pangasius	Basa Fish Fillet	Red Fish Basa Fillet S/C	Sutchi catfish *(Pangasianodon hypophthalmus*)[b]/ *Pangasius bocourti*[c]/*Pangasius krempfi*[c]/*Pangasius djambal*[ac]	Swai or Sutchi or Striped Pangasius or Tra/Basa	"Red fish" and basa refer to different species
P092	Rockfish/Snapper	Fresh Pacific Snapper Filet	Pacific Rockfish Fillet Wild-Fresh	Widow rockfish (*Sebastes entomelas*)	Rockfish, Pacific Red Snapper[d]	"Rockfish" and "Pacific snapper" refer to different species

P107	Rockfish/Snapper	Fresh Rockfish Red Snapper	Rock Fish Fillets	Darkblotched rockfish (*Sebastes crameri*)/ Northern rockfish (*Sebastes polyspinis*)/ Yellowmouth rockfish (*Sebastes reedi*)/ Vermilion rockfish (*Sebastes miniatus*)[a]	Rockfish, Pacific Red Snapper[d]	"Rockfish" and "Red snapper" refer to different species
P020	Salmon	Salmon Fillet	Fresh Salmon Fillet	Atlantic salmon *(Salmo salar)*	Salmon, Atlantic	"Atlantic" must be specified
P033	Salmon	N/A (no placard)	Salmon	Atlantic salmon *(Salmo salar)*	Salmon, Atlantic	"Atlantic" must be specified
P040	Salmon	Salmon Fillet	Salmon Fish Fillet S/C	Atlantic salmon *(Salmo salar)*	Salmon, Atlantic	"Atlantic" must be specified
P045	Salmon	Fresh Salmon Fish Fillet	Fresh Salmon Fish Fillet	Atlantic salmon *(Salmo salar)*	Salmon, Atlantic	"Atlantic" must be specified
P050	Salmon	Salmon Fillet Skin On	Salmon Fillet Skin On	Atlantic salmon *(Salmo salar)*	Salmon, Atlantic	"Atlantic" must be specified

[a]BOLD showed equivalent top matches to all species listed.
[b]Although the common name for *P. hypophthalmus* is Sutchi catfish, non-Ictaluridae members of the Siluriformes (catfish) order, cannot legally use the term "catfish" in their market name (section 403(t) of the FD&C Act (21 U.S.C. 343(t)).
[c]The FDA Seafood List does not have records for the following species: *Pangasius bocourti*, *Pangasius krempfi, Pangasius djambal,* and *Pseudocrenilabrus multicolor*.
[d]Pacific Red Snapper is considered a vernacular name when used in interstate commerce, but it is an acceptable market name in California (California Code of Regulations §103)

Table 5. Summary of COOL noncompliance for the fish samples collected in this study. Values are given as the number count.

Category	Samples collected	COOL non-compliant samples	Country of origin declaration			Production method declaration		
			Domestic (USA)	Imported	Not Stated or Unclear	Wild	Farmed	Not Stated or Unclear
Bass	7	3	0	4	Unspecified: "Korea" or "Korean" (2) Not stated (1)	6	0	Not stated (1)
Catfish	10	3	7	1	Contradictory information (1) Not stated (1)	1	8	Not stated (1)
Cod	10	0	6	4	0	10	0	0
Halibut	10	2	6	2	Contradictory information (2)	10	0	0
Mahi-mahi	6	3	0	4	Not stated (2)	3	1	Not stated (1) Unclear wording: "Born, Raised, Harvested China" (1)
Pangasius	10	2	1	7	Not stated (2)	1[a]	10[a]	0
Rockfish	6	0	2	4	0	6	0	0
Rockfish/ Snapper	2	0	1	1	0	2	0	0
Salmon	10	3	0	9	Not stated (1)	1	7	Not stated (2)

Snapper	3	1	0	2	Contradictory information (1)	1	1	Contradictory: “Farm Raised” on placard and “Wild” on label (1)
Sole	10	1	9	0	Not stated (1)	10	0	0
Swordfish	10	2	3[b]	7[b]	Contradictory information (1)	9	0	Not stated (1)
Tilapia	10	2	0	9	Not stated (1)	0	9	Unclear wording: “BRN,RAISD&HARVST CHINA” (1)
Trout	2	0	2	0	0	0	2	0
Tuna	10	5	3[b]	6[b]	Not compliant: “Tahiti” (1) Contradictory information (1)	7	0	Not stated (3)
Yellowtail	4	1	0	3	Not stated (1)	2	1	Not stated (1)
Total	120	28	40	63	19	69	39	13

[a]One sample of Pangasius listed both farm raised and wild caught as the production method. This sample was considered to be COOL compliant due to the possibility of a commingled commodity (7 CFR §60).
[b]One sample of swordfish and one sample of tuna listed USA, Mexico, and Canada as the countries of origin. These samples were considered to be COOL compliant due to the possibility of a commingled commodity (7 CFR §60).

Figure 1. Examples of COOL compliant sticker labels (a-b) and seafood counter placards (c-d) on individually packaged products. Store names have been redacted from labels.

Figure 2. Examples of (a) COOL non-compliant sticker (invalid country name) on an individually packaged product (b) COOL non-compliant placard (unclear wording regarding production method) at the seafood counter (c) COOL non-compliant placard (no country or production method) at the seafood counter (d) COOL non-compliant sticker (no country or production method) on an individually packaged product