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

A Thesis by

Priscila Liou

Chapman University

Orange, California

Schmid College of Science and Technology

Submitted in partial fulfillment of the requirements for the degree of

Master of Science in Food Science

May 2019

Committee in charge:

Rosalee Hellberg, Ph.D., Thesis Advisor

Fredric Caporaso, Ph.D.

Lilian Were, Ph.D.

The thesis of Priscila Liou is approved.

Rosalee S. Hellberg, Ph.D., Thesis Advisor
Assistant Professor
Food Science Program
Chapman University

Fredric Caporaso, Ph.D., Committee Member
Professor
Food Science Program
Chapman University

Lilian Were

Digitally signed by Lilian Were
DN: cn=Lilian Were, o=Chapman university, ou, email=were@chapman.edu, c=US
Date: 2019.05.22 14:17:09 -07'00'

Lilian Were, Ph.D., Committee Member
Associate Professor
Food Science Program
Chapman University

January 2019

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

DEDICATION

This thesis is dedicated to my family and friends who have been a part of this journey with me. To my brother, who is my favorite and only sibling. I continue to be amazed by your compassion and kindness, your commitment to excellence, and your ability to see the bigger picture during challenging times. To my dad, my grandma, and my aunt for their never ceasing love from a half a world away. To my friends for their support during my entire program, especially Julie Nguyen, Kaisa Dodge, and Michelle Xu. You will always be my sisters at heart. Most importantly, to my mom who without her love, support, and cooking, this thesis could not have been written. Thank you for supporting me in every way possible and for always being my anchor when I feel lost. You are the person I want to make proud in everything that I do.

ACKNOWLEDGEMENTS

The author wishes to thank Dr. Rosalee Hellberg for serving as thesis advisor and mentor, for her resilient patience and unwavering support, and for all the knowledge that she has shared and taught me. Dr. Lilian Were for her dedication and for always challenging me to think critically. Dr. Fredric Caporaso for his time and support as a committee member. Rachel Isaacs for her lab training and for always lending a hand when needed. Angela Banda for all her time and work for this project. Jennifer Chan, Alyssa Hardy, and AJ Silva for their support and friendship. Maggie Chung for being the best friend I could ask to take this challenging journey with – thank you for keeping a smile on my face in the most challenging times while keeping me focused. Dr. Ken 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. Chapman University Schmid College of Science and Technology for providing funding for this research.

ABSTRACT

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

by Priscila Liou

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 grocery stores. Each sample underwent DNA barcoding to determine the species. Use of an acceptable market name was 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. Among the 120 samples examined, species substitution was detected in 16 samples (13.3%) and unacceptable market names were observed for an additional 11 samples (9.2%). The category with the highest rate of species substitution was snapper (3/3), followed by 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. Overall, 25 out of the 30 grocery stores visited had at least one sample with a mislabeling error. This study revealed species mislabeling as a continuing concern in the seafood industry, especially with high-value species. Furthermore, the lack of COOL compliance among retailers is concerning and suggests a need for increased focus on these regulations.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

COOL – Country-of-Origin Labeling

E.U. – European Union

FAO – Food and Agriculture Organization of the United Nations

FDA – Food and Drug Administration

NOAA – National Oceanic and Atmospheric Administration

USDA – U.S. Department of Agriculture

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 kg of fish and shellfish were consumed per person in 2015, an increase of 0.4 kg from the previous year (NOAA, 2015). The top commercial fish consumed in the U.S. are salmon, canned tuna, tilapia, pollock, Pangasius, cod, and catfish (Delaware SeaGrant, 2018). Many fish species 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 and allergens. Mislabeling can also interfere with religious practices 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* (FDA, 2018b). However, numerous studies have reported seafood species substitution and mislabeling on the U.S. marketplace (Bosko, Foley, & Hellberg, 2018; Cline, 2012; 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 used to successfully identify fish species in numerous studies (reviewed in Hellberg, Pollack, & Hanner, 2016), and it has been adopted by the U.S. FDA for regulatory identification of fish species (Handy et al., 2011). 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 (Ratnasingham & Hebert, 2007; FDA, 2018a).

In addition to accurate species labeling, certain seafood products 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 exempted 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 96.2% of the 628 fresh/frozen seafood samples collected from 14 stores were COOL compliant (Lagasse, Love, & Smith, 2014). Among the samples examined, 1.1% did not state a country of origin, 1.9% listed multiple countries 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. Review of Literature

2.1 History of species mislabeling for fish and rise of seafood consumption

Americans consumed an estimated 7 kg of fish and shellfish per person in 2015 (NOAA, 2015). This was an increase in seafood intake of 0.4 kg from 2014 (NOAA, 2015). According to the Food and Agricultural Organization of the United Nations (FAO) (2018), global per capita seafood consumption is over 20 kg per year and about 3.2 billion people depend on seafood as a source of food. According to the Seafood Health Facts website (www.seafoodhealthfacts.org), the top commercial fish consumed in the U.S. are salmon, canned tuna, tilapia, Alaska Pollock, Pangasius, cod, and catfish. Some species such as red snapper or mahi-mahi are more limited in supply which increases their value (FDA, 2014).

2.1.1 Seafood and its susceptibility to fraud

With the rising consumption of seafood, fraudsters are using demand as an opportunity for economic gain (Hellberg & Morrissey, 2011). Fraudsters can profit from selling low-value fish substituted and mislabeled as high-value fish. Furthermore, since it is difficult to identify different species of fish based purely on appearance, many consumers are deceived. As **Figure 1** shows, many fish fillets are similar in appearance.

The similarity can become more confusing with further processing such as when fish are in products like poke and sushi.

Figure 1. Filleted white fish from a local grocery store – a) farm raised tilapia from Malaysia; b) wild caught Alaska cod from USA.

Table 1 provides examples of some higher-value fish species that have been substituted with cheaper fish species. Intentional mislabeling is illegal under the Food,

Drug, and Cosmetic (FD&C) Act (21. U.S.C. 343(a)(1)) (FDA, 2017). A series of market surveys conducted by the nonprofit organization Oceana revealed 55% fish species substitution in Southern California and 33% species substitution nationwide from 2010-2012 (Warner et al., 2012, 2013). The results were greater than the 25% mislabeling reported for North American seafood by Wong and Hanner (2008). However, Wong and Hanner (2008) tested fewer samples, 96 compared to 1,215, and they tested samples from Canada in addition to the United States.

Table 1. Higher-value fish species that have been known to be substituted with a lower-value fish species (FDA, 2014)

Higher-Value Fish Species	**Lower-Value Fish Species**
Red Snapper (*Lutjanus campechanus)*	Various Snappers and Rockfish
Mahi Mahi	Yellowtail
Swordfish	Mako Shark
Dover Sole	Arrowtooth Flounder
Cod	Alaska Pollock
Halibut	Sea Bass
Salmon	Steelhead Trout
Wild Caught Salmon	Farm Raised Salmon

In addition to economic deception, species mislabeling can lead to health hazards (**Table 2**). For example, consumption of escolar, which has been mislabeled as white tuna, sea bass, or grouper, may cause gastrointestinal discomfort in the form of diarrhea and cramps (Unicomb, Kirk, Yohannes, Dalton, & Halliday, 2002; Yancy et al., 2008). The FDA recommends that consumers avoid consuming escolar due to the risks (FDA, 2011). In addition, pufferfish being mislabeled as monkfish may cause paralysis and potential death due to tetrodotoxin (Cohen et al., 2009). In 2007, two cases of tetrodotoxin poisoning occurred from the individuals eating home-cooked pufferfish sold as monkfish. Although both the retailer and supplier denied selling pufferfish, DNA analysis and visual inspection proved that the labeled monkfish was illegally imported pufferfish.

Histamine is indicative of how long a fish has been decomposing as it is only formed post-mortem in species such as tuna and mahi-mahi (FDA, 2015). Histamine is heat resistant and can cause scromboid poisoning (FDA, 2011). The potential for increased histamine formation occurs when scrombotoxin-forming fish muscle is further processed and more surface-to-volume ratio is exposed, such as with minced tuna (FDA, 2011). Species substitution involving these types of fish can lead to unexpected cases of scombroid poisoning (Table 2).

Ciguatera fish poisoning (CFP) derives from fish eating toxic marine algae or from fish that have eaten any fish that consumed toxic marine algae (FDA, 2016). CFP then manifests in humans with symptoms of nausea, vomiting, diarrhea, possible numbness and tingling, itchiness, joint pain, and others. Symptoms may last from a few days to months or years (FDA, 2016). CFP can result from consumption of fish with

accumulated ciguatoxins that have been labeled and sold as other fish species. The possible health hazards discussed above indicate how dangerous species mislabeling can be. Without proper labeling, consumers cannot make informed decisions about what species to avoid or take necessary precautions. Without knowing the true identity of some of these fish species, some consumers may fall ill or even die from overconsumption or improper handling.

Table 2. Health hazards related to species mislabeling (Cohen et al., 2009; Unicomb et al., 2002)

Labeled Species	**Identified Species**	**Health Hazards of Identified Species**	**Documented Cases**
Sea Bass, Grouper, White Tuna	Escolar	Gempylotoxin and histamine	N/A
Monkfish	Pufferfish	Tetrodotoxin	2007 – woman hospitalized with neuro symptoms after soup ingestion (Cohen et al., 2009)
Whitefish	Amberjack	Histamine and ciguatera fish poisoning	N/A
Kingfish	Spanish Mackerel	Histamine and ciguatera fish poisoning	N/A
Grouper	Basa	Environmental hazards, chemical contaminants, and pesticides in the water from which basa may have been gathered	

Table 3 depicts the acceptable market names of some common fish species, according to the FDA's *Seafood List*. The FDA recommends that fish be labeled using an acceptable market name provided in *The Seafood List* to provide an appropriate, statement of identity that is not misleading (FDA, 2012b). Fish is a unique category of foods where a name is often shared among multiple species (FDA, 2012b). For example, three different species of flounder can all be marketed as "flounder" as an acceptable market name (**Table 3**). Therefore, FDA's *The Seafood List* includes both an acceptable market name that is sometimes a more general term and a common name where consumers can get a level of specificity. Instead of "flounder," the three different species can be marketed using their common names of "tropical flounder," "Mexican flounder," and "Pantagonian flounder" (**Table 3**). It is important to note that the acceptable market names provided in *The Seafood List* are suggestions in order to avoid mislabeling; however, these names are not required to be used by industry unless they are associated with a specific law.

Table 3. Acceptable market names associated with some common fish species. Adapted from FDA, 2018b.

Acceptable Market Name	**Common Name**	**Scientific Name**
Atlantic Salmon	Atlantic Salmon	*Salmo salar*
Sockeye OR Red OR Blueback Salmon	Sockeye Salmon	*Oncorhynchus nerka*
Chinook OR King OR Spring Salmon	Chinook Salmon	*Oncorhynchus tshawytscha*
Halibut	Pacific AND Atlantic Halibut	*Hippoglossus hippoglossus* and *Hippoglossus stenolepis* respectively
Flounder	Tropical AND Mexican AND Pantagonian Flounder	*Bothus mancus* and *Cyclopsetta chittendeni* and *Paralichthys patagonicus*
Sole	Mud AND Narrowbanded AND Scrawled Sole	*Austroglossus pectoralis* and *Synclidopus macleayanus* and *Trinectes inscriptus*
Flounder OR Sole	Yellowtail Flounder AND Blackback	*Limanda ferruginea* and *Psuedopleuronectes americanus*
Tilapia	Nile AND Mango AND Redbreast Tilapia	*Oreochromis niloticus* and *Sarotherodon galilaeus galilaeus* and *Tilapia rendalli*
Catfish	White AND Yaqui AND Flathead Catfish	*Ameiurus catus* and *Ictalurus pricei* and *Pylodictis olivaris*
Pollock	Pollock	*Pollachius virens*
Cod OR Alaska Cod	Pacific Cod	*Gadus microcephalus*
Cod	Polar AND Atlantic AND Maori Cod	*Arctogadus glacialis* and *Gadus morhua* and *Paranotothenia magellanica*
Snapper	Black AND Yellowstripe AND Pacific Snapper	*Apsilus dentatus* and *Etelis coruscans* and *Lutjanus peru*
Red Snapper OR Snapper	Red Snapper	*Lutjanus campechanus*

2.1.2 Seafood mislabeling studies

Numerous studies have been conducted to examine species substitution in seafood around the world. A few studies are discussed in this section. In the first published study to use DNA barcoding to reveal species mislabeling, Wong and Hanner (2008) collected 96 fish and seafood samples from commercial markets and restaurants in both the U.S. and Canada. The samples collected were either raw or cooked. Of the 91 samples successfully sequenced, 23 were suspected of being mislabeled (25%). Three samples identified as mislabeled represented differences between acceptable market names between the FDA's *Seafood List* and the Canadian Food Inspection Agency (CFIA) list. "Red snapper" had the highest mislabeling rate as seven of the nine samples were not identified as *Lutjanus campechanus*. Mislabeling was also found with halibut and sea bass samples.

A U.S. study conducted from 2010-2012 found a mislabeling percentage of 33% from 1,213 samples collected from 674 retail outlets in 21 states (Warner et al., 2013). Forty-four percent of the all retail outlets sold mislabeled fish. Seventy-six percent of all sushi venues tested had mislabeled products while only 18% of the grocery stores sold mislabeled fish. Warner et al. (2013) found that restaurants had a higher mislabeling percentage than grocery stores. Of all the samples, snapper and tuna had the highest mislabeling rates at 87% and 59%, respectively. Halibut, grouper, cod, and Chilean sea bass were also mislabeled 19-38% of the time whereas salmon had a mislabeling rate of 7%. Among samples collected in the Southern California region, Warner et al. (2012) found that 55% of the samples from sushi restaurants, grocery stores, and restaurants were mislabeled.

In an FDA survey of seafood labeling at the wholesale level, 174 lots of fish were tested across three sampling efforts (FDA, 2012a). Sampling efforts targeted high risk categories of mislabeling and/or substitution. A 15% mislabeling percentage was reported, with the snapper and grouper categories comprising the majority of the mislabeled lots (25/26). Testing occurred across 14 states.

A study conducted in Los Angeles, CA, from 2012 to 2015 reported 47% and 42% species mislabeling in sushi restaurants and upscale grocery stores, respectively (Willette et al., 2017). Samples from both sushi restaurants and grocery stores were described as sushi-grade fillets with nine categories of fish targeted. The fish with the highest percentage of mislabeling in restaurants were halibut, red snapper, yellowtail, and yellowfin tuna, in descending order. All samples of halibut and red snapper tested were mislabeled; 93% of yellowtail was mislabeled; and about 50% of yellowfin tuna was mislabeled. Similarly, Willette et al. (2017) found the highest percentage of mislabeling in grocery stores with red snapper, yellowfin tuna, and yellowtail.

In another study, Khaksar et al. (2015) tested fresh fish and seafood samples from three U.S. cities – New York City (NY), Austin (TX), and San Francisco (CA) - and found a 16.3% mislabeling percentage out of 172 samples. Most samples (78.5%) were collected from sushi restaurants, while the remaining samples were from wholesalers/retailers in the San Francisco area. The authors found that the restaurants had a 14.4% mislabeling rate compared to the 2.2% from retailers. They hypothesized the reason behind this finding was brand protection and increased consumer transparency.

Nagalakshmi et al. (2016) tested 100 samples of various fish (fresh, frozen, canned, ready to cook, and ready to eat) in India collected from fishmongers,

supermarkets, and restaurants. The authors reported a mislabeling rate of 22%. Similar to the previous studies, restaurants had a higher mislabeling rate (32%) than local markets (13%) and supermarkets (9%). Furthermore, certain species known to be delicacies were substituted for lower-value species such as “rawas” (*Eleutheronema tetradactylum*) for “bronze croaker” (*Otolithoides biauritus*). The price difference between the two species was about $4.40-6.60/kg.

From 2013-2016, 354 seafood samples were collected using a CFIA sampling plan that did not target specific species or producers (Shehata, Naaum, Garduno, & Hanner, 2018). Samples were non-processed or minimally-processed (e.g. salted) finfish in whole or fillet form. Of the 330 successfully tested samples, 49 were mislabeled (14.8%). Red snapper continued to have a high mislabeling rate (7/9) and one mislabeled sample was identified as endangered on the IUCN Red List.

In a study in Orange County, CA, Bosko et al. (2018) collected 80 catfish samples from July to August 2016. Half of the samples were restaurant dishes and the other half were fresh/frozen fish. Seven of the 80 samples (9%) were mislabeled due to species substitution with all seven samples identified as Pangasiidae species instead of Ictaluridae species. The rate of species substitution was higher among restaurant dishes (12.5%) compared to the fresh/frozen products (5%). The two mislabeled fresh/frozen products were fillets and the authors found that fillets had the highest average price for fresh/frozen products ($3.63 ± 1.27 per 267 g serving) compared to the whole catfish, nuggets, or cuts (<$2.00 per 267 g serving).

In another study, Hu et al. (2018) acquired 285 fish samples from September 2017 and February 2018 from grocery stores, sushi bars, and non-sushi restaurants in metro

Vancouver. Non-sushi restaurants had the highest rate of mislabeling (28%), followed by grocery stores (24%), and sushi bars (22%). Similar to previous studies, snapper, yellowtail, cod, halibut, and sea bass continued to have the highest rates of species substitution.

2.2 Methods of detection for species substitution

Species authentication can be carried out using a variety of methods, including morphology, protein- and DNA-based methods. Morphological identification can be carried out using taxonomy data typically from experts (Naaum & Hanner, 2016). However, morphological techniques are often not practical for use with commercial fish products due to the removal of taxonomic features during processing. Protein-based methods use unique proteins to determine species identification whereas DNA-based methods use genetic markers to identify species (Bosko et al., 2018). Protein-based methods also require the storage of standards at low temperatures over time, which can lead to degradation. Furthermore, proteins in food are more vulnerable to degradation when the food is cooked or heavily processed. Overall, DNA-based methods are more robust than protein-based methods in regards to storing standards and providing species identification (Nagalakshmi et al., 2016). Commonly used DNA-based methods include species-specific polymerase chain reaction (PCR), restriction fragment length polymorphism (RFLP), biosensors, microarrays, and DNA sequencing (Naaum & Hanner, 2016).

2.2.1 Protein-based methods of detection for species mislabeling

Protein-based methods for species identification include enzyme-linked immunosorbent assay (ELISA) and isoelectric focusing (IEF). Protein-based methods are

reliable for testing fresh, lightly processed fish, but are less sensitive than DNA-based methods, have limited applicability, and use a targeted approach (Bosko et al., 2018). IEF is an official method for raw fish identification that was used by Wang and Hsieh (2016) to differentiate between two Pangasius species (*Pangasius hypothalamus* and *Pangasius bocourti*). IEF differentiated the two species by comparing species-specific protein banding patterns to banding patterns of Pangasius positive samples. However, when four samples presented a different banding pattern than the Pangasius positive samples, the species could not be determined. Specifically, the four samples were restaurant "grouper" samples that did not test positive for Pangasius or grouper. This indicated that the samples could have been another species of grouper, cross-bred grouper, or a non-grouper species (Wang & Hsieh, 2016). The study also used iELISA and lateral flow strip assays to verify their data.

2.2.2 DNA-based methods of detection for species mislabeling

DNA-based methods are widely implemented in species authentication studies due to their accuracy and increased ease of use (Naaum & Hanner, 2016). The methods focus on extracting the genetic information needed to obtain the correct species identification. DNA is a stable molecule that is found in almost all cells and has been shown to survive strenuous processing (Naaum & Hanner, 2016). Choosing the correct DNA-based method will depend on the cost, equipment available, and processing of the sample, among other factors. The processing of the sample is one of the most important factors since greater processing can lead to fragmentation of DNA and may require a method that targets short regions of DNA. However, nucleic acids are very stable and can survive most industrial processes such as high heat and pressure.

Species-specific PCR has been used for species identification in seafood studies focused on individual species (for example, Bosko et al., 2018; Eischeid, 2019; Hulley, Tharmalingam, Zarnke, & Boreham, 2019; Marín, Fujimoto, & Arai, 2013; Tetzlaff & Mäde, 2017). PCR is often used to quantify results or to test samples that have undergone intense processing (Naaum & Hanner, 2016). For example, Bosko et al. (2018) tested samples that were fried, steamed, and grilled. In the last two decades, real-time PCR has been used more frequently by scientists as a method of identification since it saves time and decreases error by eliminating a post-PCR step (Naaum & Hanner, 2016). However, it is a targeted method for species identification and does not test simultaneously for a broad range of species.

RFLP is another DNA-based method of identification that works by creating restriction profiles of species by cutting the DNA at different lengths (Naaum & Hanner, 2016). One study used PCR-RFLP targeting the cytochrome *b* gene to identify 10 white fish species (Dooley, Sage, Clarke, Brown, & Garrett, 2005). Another study used a modified version of the previous PCR-RFLP method to target the cytochrome *c* oxidase I (COI) gene (Handy et al., 2017). However, DNA barcoding proved more robust for certain species such as *Sebastes* spp. and *Lutjanus* spp (Handy et al., 2017). Other drawbacks include the need for a post-PCR incubation step, use of gel electrophoresis, and errors associated with interpretation of results and reproducibility (Naaum & Hanner, 2016).

Biosensors and microarrays have been identified as newer methods, but there is a lack of studies using these methods for species identification (Naaum & Hanner, 2016). Scientists have previously used biosensors for determining fish gender (Rahman et al.,

2017). In addition, a DNA microarray has been used as a tool for Tc1 transposon sequence analysis in fish genomes (Wenne et al., 2011). As these methods become more developed, they may be more widely used in species identification studies.

A commonly used method for fish species identification is DNA sequencing (Naaum & Hanner, 2016). The chain termination sequencing method, also known as Sanger sequencing, provides the greatest amount of genetic information from a sample among the methods discussed here because it reveals the actual nucleotide sequence of the DNA (Naaum & Hanner, 2016). The method is used after PCR amplification – a sequence is produced from the amplicon using Sanger sequencing. This sequence can then be used to search a database of sequences from known fish species (Naaum & Hanner, 2016). DNA sequencing is a specific and accurate method in detecting species substitution in fish (Khaksar et al., 2015; Wong & Hanner, 2008).

2.2.3 DNA barcoding as a method of fish species identification

DNA barcoding is a sequencing-based method that uses a short, standardized genetic marker to identify the sample to a certain species (Hebert, Cywinska, Ball, & deWaard, 2003). To ensure accurate species identification, the target marker and the availability of reference libraries need to be considered (Hellberg et al., 2016). **Table 4** summarizes the main advantages and limitations of two common target markers; cytochrome *b* (cyt *b*) and cytochrome oxidase I (COI), used in seafood identification in DNA sequencing. 16S rRNA is another marker used for seafood species identification; however, it has a lower rate of divergence compared to the two previously mentioned markers (Hellberg et al., 2016). Furthermore, sequence alignments can be complicated due to insertions and deletions in the gene coding for ribosomal DNA (Hellberg et al.,

2016). The predominant genetic marker used for DNA barcoding of animal species is the gene coding for COI. The COI mitochondrial gene has been established as the sequence for animal identification due to its high variability and conservation of PCR primer sites (Stern et al., 2017). DNA is extracted from tissue samples and amplified using PCR before it is sequenced. Following sequencing, the sequence is compared to sequences of known species in a database to enable species identification. The main database used for the identification of DNA barcodes is the Barcode of Life Database (BOLD). BOLD contains over three million COI DNA barcodes representing close to 200,000 animal species (Ratnasingham & Hebert, 2007).

Table 4. A comparison of two target markers for DNA sequencing (Hellberg et al., 2016)

Genetic Target	Advantage	Limitation
Cyt *b*	Universal primers available	464 base pair (bp) region compared to COI's 650 bp region – less data gathered
	Can target shorter fragment lengths	Some difficulty differentiating closely related species
		False identification for a hybrid species since mtDNA is always inherited from the maternal side
		Unable to differentiate species that COI could
COI	Strong phylogenetic signal	Some difficulty differentiating closely related species
	Range of universal primers available	False identification for a hybrid species since mtDNA is always inherited from the maternal side
	FDA's chosen method for regulatory fish testing	
	Extensive research studies using this method	
	Growing popularity of it becoming a global standard method	
	Reliability of reference libraries	

One of the first studies using DNA barcoding to detect species substitution in fish was published by Wong & Hanner (2008). Since then, DNA barcoding has been used to successfully verify identification of fish species by multiple scientists in various studies (Hu et al., 2018; Khaksar et al., 2015; Mitchell & Hellberg, 2016; Nagalakshmi et al., 2016; Shehata et al., 2018; Warner et al., 2013; Willette et al., 2017; Wong & Hanner,

2008) and is now the standard test by the U.S. FDA for seafood identification (Handy et al., 2011). DNA barcode data has also been collected and included in FDA's Regulatory Fish Encyclopedia as a resource for species mislabeling and substitution (FDA, 2018a). Although DNA barcoding is accurate and robust, it is a relatively time-consuming and labor-intensive method that does not always work well in industry settings.

2.3 COOL regulations as pertains to fish labeling

COOL is a labeling law that requires retailers licensed under the Perishable Agriculture Commodities Act (PACA) to provide sourcing information in regards to the geographic origin of meat, fresh and frozen fruits and vegetables, peanuts, pecans, macadamia nuts, ginseng, fish, and shellfish to consumers (USDA, 2017a). Agricultural Marketing Service (AMS) is the regulatory agency that monitors and enforces the COOL regulations (USDA, 2017a). Additionally, unless exempted by law, foreign articles imported into the U.S. must be labeled with a proper country of origin according to 19 CFR §134.11. If a product originates from multiple countries, all countries must be listed (USDA, 2017b). All fresh and frozen fish fillets, steaks, and nuggets, either wild or farm raised, must follow COOL regulations (USDA, 2017b).

The COOL regulations state that retailers must inform consumers of the country of origin information and production method (wild-caught or farm-raised). However, the regulations do not stipulate any size or font of how the information must be displayed (USDA, 2017b). While the information can be displayed in a variety of locations such as on a placard, sign, label, sticker, band, or twist tie, it must be legible and placed in a conspicuous location. The regulations also dictate that abbreviations for countries are not acceptable unless the codes cannot be mistaken for any other country or are common. For

instance, “P.R. China” is suitable for “China” and “Holland” is acceptable for the Netherlands. However, “America” would be not be acceptable as it can mean North America, Central America, or South America (USDA, 2017b). Furthermore, COOL regulations prohibit phrases such as “or,” “may contain,” and “and/or” to prevent confusion to consumers (USDA, 2017b).

The E.U. is the largest single market for fish imports (FAO, 2018). The U.S., however, is the world’s largest single importer of fish and fishery products. Japan and China rank second and third, respectively (FAO, 2018). The combination of the imports to the E.U., the U.S., and Japan account for 64% of the total value of the world imports of fish and fishery products (FAO, 2018). The fish and fishery product imports in the U.S. (over $20 million) is a stark contrast to its export value of $5.8 million (FAO, 2018). Overall, about 90% of the seafood consumed in the U.S. is imported (NOAA, 2017). Imported products often have complex supply chains that may involve transit through multiple countries. Lack of regulations, government instability, and different local values are important factors that may affect the quality and safety of products coming from developing countries. For example, lack of regulations translate to poor manufacturing practices and government instability can equate to an unstable economy and create motivation for fraudsters to earn money. Therefore, it is crucial that consumers are provided with accurate COOL information. COOL has been found to be an important consideration to shoppers in grocery stores as a signal of food safety (Lagasse et al., 2014).

In addition, production method such as whether a fish was farm-raised or wild-caught should also be stated. Advertisements for wild-caught fish were noted to use

brighter colors such as blue, green, and yellow along with “fresh” or “all natural” descriptors (Lagasse et al., 2014). This marketing may have trained consumers to view these descriptors as indicators of food safety and quality. Fraudsters can use this preference to mislead consumers through mislabeling of the production method.

Only one study has been published regarding COOL for seafood in Southern California (Bosko et al., 2018). Bosko et al. (2018) reported that 59% of 32 catfish products collected from grocery stores were not compliant with COOL regulations. Among the non-compliant samples, 50% were missing production method information, 31% samples did not state a country of origin, and 22% had neither a production method or country of origin stated (Bosko et al., 2018). Also, two of the COOL non-compliant fillets collected from grocery stores were further mislabeled on the basis of species.

Another study that tested COOL compliance was carried out by Lagasse et al. (2014). Lagasse et al. (2014) performed a study in Baltimore, MD, that reported 96.2% of samples examined were COOL compliant. They took pictures of 628 samples (non-packaged fresh, packaged fresh, and frozen) from 14 stores. Each store was visited at least twice. Of all samples, 1.9% were not COOL compliant in terms of stating a country of origin, 2.7% did not state a production method, and 1.1% listed neither country of origin or production method. As for the most commonly sold seafood (salmon, tilapia, catfish, and shrimp), salmon was the only fish to have a portion (3.4%) of its 87 samples to have no production method stated. As for country of origin, 8.0% of salmon, 4.7% of tilapia, and 4.5% of catfish samples were not COOL compliant.

The USDA Agricultural Marketing Service (AMS) conducts COOL compliance reviews in all 50 states, with the latest results published online for their 2016 surveillance

reviews (https://www.ams.usda.gov/rules-regulations/cool/compliance-enforcement). The 2016 surveillance data revealed 10% COOL noncompliance for fish and shellfish sold at the retail level, while the 2015 surveillance data revealed 7.4% COOL noncompliance. Each year, more than 75,000 fish and shellfish products from over 3,000 retail store facilities were examined (K. Becker, personal communication, June 21, 2017). When the data for both years was combined, about 45% of noncompliance findings were due to products missing a country of origin and 55% were due to products missing production method information (K. Becker, personal communication, June 21, 2017).

2.4 Rationale and significance

This study provides current information to regulators and consumers about fish species substitution and COOL compliance in Southern California. The overall goal was to test fish fillets sold in Southern California grocery stores for species authentication, use of acceptable market names, and COOL compliance. This goal was addressed with the following specific aims:

I) Collect 120 fresh/frozen fish samples from 30 grocery stores in Southern California

II) Observe and note COOL compliance and use of acceptable market names for fish samples.

III) Identify the species of each fish sample using DNA barcoding.

The *significance* of this study is that seafood fraud is a continuous problem in the United States and that more information on labeling trends was needed. Not only can mislabeling be a source of economic adulteration, but there have been health risks associated with species mislabeling. The results of this study provide information

regarding whether retailers are correctly reporting species and complying with COOL regulations to provide safe seafood to their consumers.

3. Materials and Methods

3.1 Sample collection

A total of 120 fresh or thawed (previously frozen) fish fillets were collected from 30 grocery stores in Orange County, CA. Fifteen categories of fish were targeted based on their availability at grocery stores: salmon, cod, tuna, halibut, tilapia, catfish, Pangasius, rockfish, snapper, sole, trout, swordfish, mahi-mahi, bass, and yellowtail. An additional category named "rockfish/snapper" was added due to the collection of two 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 at the same store. 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 and species labeling were noted at the time of purchase (e.g., on placards, stickers, signs, labels, etc.) with the exact wording recorded. 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 coolers 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 DNA extraction. The remaining sample was preserved at -80°C.

3.2 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 -80°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.

3.3 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. 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 5), 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.

Table 5. 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

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 5), and 2.0 μL of DNA template. Cycling conditions were

95ºC for 5 min; followed by 35 cycles of 94 ºC for 40 s, 46 ºC for 1 min, and 72 ºC for 30 s; with a final extension of 72 ºC 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 5), and 3.0 μL of DNA template. Cycling conditions were 94ºC for 2 min; followed by 35 cycles of 94 ºC for 30 s, 49 ºC for 40 s, and 72 ºC for 1 min; with a final extension of 72 ºC 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).

3.4 DNA sequence analysis

The 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, 2018b). For species not listed in *The Seafood List*, FishBase was used to determine the common names (FishBase, 2018).

4. Results and Discussion

4.1 DNA barcoding results

All of the 120 fish fillets collected were successfully 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. The majority of samples (n = 116) were successfully 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, 81 were identified to the species level (i.e., showed a top match to a single species in BOLD). All

samples of bass, catfish, salmon, snapper, sole, swordfish, yellowtail and most samples of cod, halibut, mahi-mahi, rockfish were identified to the species level. An additional 24 samples were identified to the genus level (i.e., showed a top match to multiple species from the same genus), and 15 samples showed top matches to multiple species in different genera. The majority of the tuna and tilapia samples were identified to the genus level, along with a few samples of halibut, rockfish, trout, and mahi-mahi. 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). In the case of the tilapia samples, most of the sequences had top matches to *Oreochromis* hybrids and therefore could not be identified at the species level. Samples with top matches from multiple genera were primarily from the Pangasius (n = 9) and cod (n = 5) categories, with one sample of tilapia. The Pangasius samples showed top matches to both *Pangasianodon* and *Pangasius* genera while the cod samples showed equivalent matches to both *Gadus* and *Boreogadus* genera. The tilapia sample had top matches to *Oreochromis* and *Pseudocrenilabrus*.

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 alalonga*) (n = 1)], southern bluefin tuna [(*Thunnus maccoyii*) (n = 1)], and *Thunnus* sp. (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).

4.2 Species substitution

Species substitution was detected in 16 of the 120 fish fillets (13.3%) examined in this study (Table 6). Among the 16 categories of fish tested, seven had at least one sample with species substitution (Figure 2). 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. Previous market surveys in the U.S. also found relatively high rates of mislabeling among snapper, halibut, and cod, and yellowtail products (Hu et al., 2018; Khaksar et al., 2015; Shehata et al., 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.

Table 6. 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
29	Bass	Seabass (Patagonian toothfish)	Seabass (Patagonian Tooth Fish)	Patagonian toothfish (*Dissostichus eleginoides*)	88.18	Antarctic toothfish (*Dissostichus mawsoni*)
101	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*)
1	Cod	Fresh Wild Caught Pacific Cod Fillets	True Cod Fillet Fresh	Pacific cod (*Gadus microcephalus*)	30.86	Atlantic cod (*Gadus morhua*)
31	Cod	Pacific Cod	Pacific Cod Fillet	Pacific cod (*Gadus microcephalus*)	33.07	Atlantic cod (*Gadus morhua*)
63	Cod	Rock Cod Fillet	Fillet of Rock Cod	Rock cod (*Lotella rhacina* or *Pseudophycis barbata*)	8.82	Redbanded rockfish (*Sebastes babcocki*)
61	Halibut	Fresh Halibut Steak	Halibut Steak	Atlantic halibut (*Hippoglossus hippoglossus*) or Pacific halibut (*Hippoglossus stenolepis*)	15.42	California flounder (*Paralichthys californicus*)
65	Halibut	Halibut Steak	Halibut Steak	Atlantic halibut (*Hippoglossus hippoglossus*) or Pacific halibut (*Hippoglossus stenolepis*)	15.43	California flounder (*Paralichthys californicus*)
69	Halibut	Halibut Steak	Halibut Steak	Atlantic halibut (*Hippoglossus hippoglossus*) or Pacific halibut (*Hippoglossus stenolepis*)	24.25	California flounder (*Paralichthys californicus*)
99	Halibut	Fresh Central Pacific Halibut Fillet	Fresh Central Pacific Halibut Fillet	Pacific halibut (*Hippoglossus stenolepis*)	61.73	California flounder (*Paralichthys californicus*)
47	Pangasius	Frozen Red Swai Fillet	Frozen Red Swai Fillet	Sutchi catfish (*Pangasianodon hypophthalmus*)	8.82	Blue-spotted stingray (*Neotrygon kuhlii*)

19	Snapper	Red Snapper Fillet	Whole Clean Red Snapper Fresh/Wild	Red snapper (*Lutjanus campechanus*)	13.19	Blackspotted rockfish (*Sebastes melanostictus*)
117	Snapper	N/A (no placard)	Fresh Red Snapper Sashimi	Red snapper (*Lutjanus campechanus*)	132.28	Madai (*Pagrus major*)
118	Snapper	N/A (no placard)	Premium Red Snapper	Red snapper (*Lutjanus campechanus*)	154.32	Madai (*Pagrus major*)
74	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*)
35	Yellowtail	N/A (no placard)	Sushi Yellowtail	Yellowtail (*Seriola lalandi*)	55.12	Buri (*Seriola quinqueradiata*)
104	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

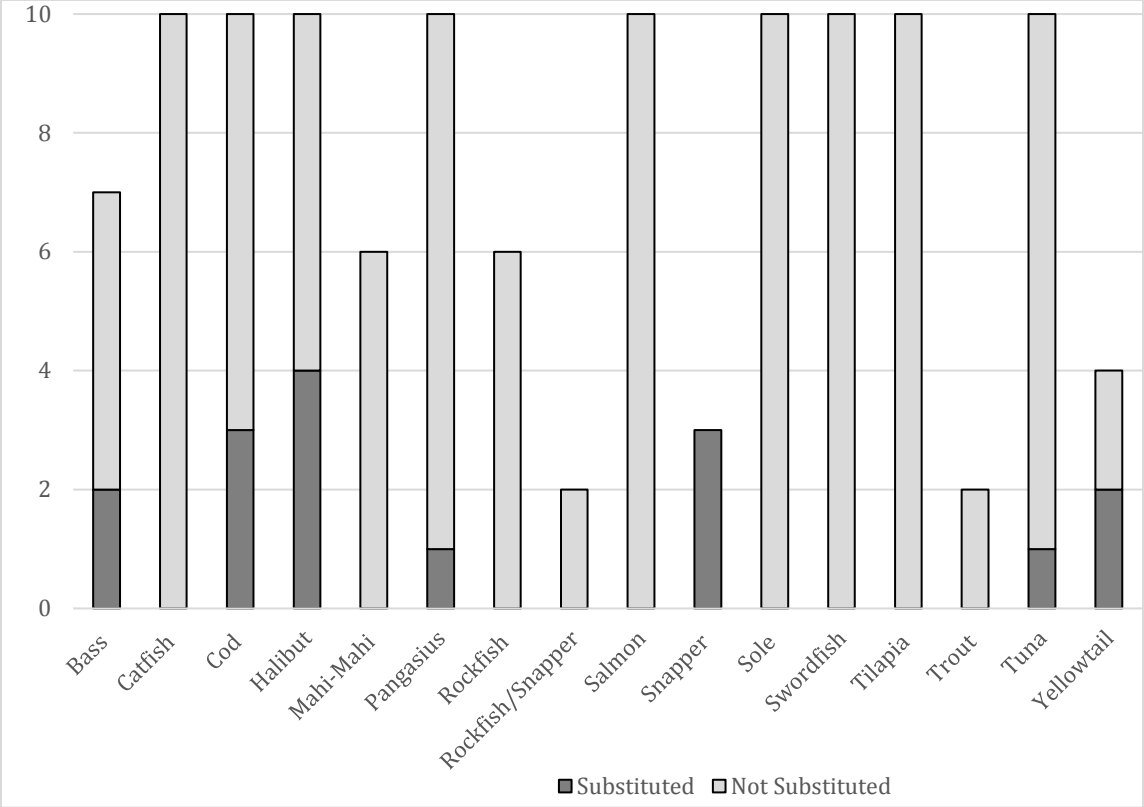

Figure 2. Proportion of samples with species substitution detected within each fish category. The "Rockfish/Snapper" category refers to samples that contained references to both rockfish and snapper on the label.

According to *The Seafood List*, the name "red snapper" is only acceptable for *Lutjanus campechanus* (FDA, 2018b). However, none of the fillets advertised as "red snapper" in this study were identified as *L. campechanus* (Tables 6-7). As shown in Table 6, 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 vermillion 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). 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 mislabeling (93%) for “red snapper”, with samples identified as madai, as well as numerous species of rockfish. 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 6). 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 6). 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 6, 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 Pangasius, tuna, and yellowtail categories each had one sample found to be substituted (Table 6). 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 this sample. Similarly, 24 out of 26 “yellowtail” samples tested by Warner et al. (2013) were identified 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.

4.3 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,

2018b). Among the 120 samples, 11 samples from 10 stores were mislabeled due to the use of an unacceptable market name (Table 7). 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). Other categories with unacceptable market names included 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 mentioned above, "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." "Red fish" appears as a vernacular name for a number of species according to *The Seafood List*. 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.”

Table 7. 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
85	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*
103	Cod	N/A (no placard)	Black Cod Kirimi	Sablefish (*Anoplopoma fimbria*)	Sablefish	Black cod is a vernacular name for sablefish
13	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
39	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
92	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

107	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
20	Salmon	Salmon Fillet	Fresh Salmon Fillet	Atlantic salmon *(Salmo salar)*	Salmon, Atlantic	"Atlantic" must be specified
33	Salmon	N/A (no placard)	Salmon	Atlantic salmon *(Salmo salar)*	Salmon, Atlantic	"Atlantic" must be specified
40	Salmon	Salmon Fillet	Salmon Fish Fillet S/C	Atlantic salmon *(Salmo salar)*	Salmon, Atlantic	"Atlantic" must be specified
45	Salmon	Fresh Salmon Fish Fillet	Fresh Salmon Fish Fillet	Atlantic salmon *(Salmo salar)*	Salmon, Atlantic	"Atlantic" must be specified
50	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)

4.4 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. COOL noncompliance was observed for 28 of the 120 samples (23.3%) in this study (Table 8). 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). COOL noncompliance was found for at least one sample from 15 of the 30 stores (50.0%) sampled.

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) 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.

Table 8. Summary of COOL noncompliance for the fish samples collected in this study. Values are given as the number count.

Category	Number of 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).

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” 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 that were technically compliant with COOL 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 fresh/frozen seafood products examined in their study. However, their samples were collected from only eight retail outlets compared to 30 outlets in this study. 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 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.

When considering all forms of mislabeling investigated in this study, 47 of the 120 samples (39.2%) had at least one labeling error. Eight samples exhibited both species mislabeling and COOL noncompliance. 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 halibut (2/7), bass (1/7), Pangasius (1/7), snapper (1/3), tuna (1/7), and yellowtail (1/7). Among the 30 stores sampled, 24 stores (80.0%) had at least one incidence of species mislabeling or COOL noncompliance.

5. Conclusion

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 (e.g. halibut and bass) substituted with lower-value species were detected in this study. 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. Noncompliant 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 assessing food safety practices, promoting seafood conservation, and allowing consumers to make informed choices. 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.

6. References

Bosko, S., Foley, D., & Hellberg, R. (2018). Species substitution and country of origin labeling of catfish products on the U.S. commercial market. *Aquaculture*, *495*, 715-720.

Cline, E. (2012). Marketplace substitution of Atlantic salmon for Pacific salmon in Washington State detected by DNA barcoding. *Food Research International*, *45*, 388-393.

Cohen, N. J., Deeds, J. R., Wong, E. S., Hanner, R. H., Yancy, H. F., White, K. D., . . . Gerber, S. I. (2009). Public health response to puffer fish (Tetrodotoxin) poisoning from mislabeled product. *Journal of Food Protection*, *72*(4), 810-817.

Collette, B., Acero, A., Amorim, A. F., Boustany, A., Canales Ramirez, C., Cardenas, G.,

. . . Yanez, E. (2011). *Thunnus albacares*. The IUCN Red List of Threatened Species. http://dx.doi.org/10.2305/IUCN.UK.2011-2.RLTS.T21857A9327139.en Accessed 2 April 2019.

Collette, B., Chang, S.-K., Di Natale, A., Fox, W., Juan Jorda, M., Miyabe, N., . . . Wang, S. (2011). *Thunnus maccoyii*. The IUCN Red List of Threatened Species. http://dx.doi.org/10.2305/IUCN.UK.2011-2.RLTS.T21858A9328286.en Accessed 2 April 2019.

Delaware Sea Grant. (2018). Seafood Health Facts. www.seafoodhealthfacts.org Accessed 26 June 2018.

Dooley, J., Sage, H., Clarke, M., Brown, H., & Garrett, S. (2005). Fish species identification using PCR-RFLP analysis and lab-on-a-chip capillary electrophoresis: application to detect white fish species in food products and an interlaboratory study. *Journal of Agricultural and Food Chemistry*, *53*(9), 3348-3357.

Eischeid, A. C. (2019). A method to detect allergenic fish, specifically cod and pollock, using quantitative real-time PCR and COI DNA barcoding sequences. *Journal of the Science of Food and Agriculture*, *99*(5), 2641-2645.

FAO. (2010). Fishery and Aquaculture Country Profiles: The Republic of Iceland. http://www.fao.org/fishery/facp/ISL/en#CountrySector-ProductionSector Accessed 16 January 2019.

FAO. (2018). The State of World Fisheries and Aquaculture. http://www.fao.org/3/I9540EN/i9540en.pdf Accessed 4 April 2019.

FDA. (2011). Fish and Fishery Products Hazards and Controls Guidance.

https://www.fda.gov/media/80637/download Accessed 12 December 2017.

FDA. (2012a). FY12 — CFSAN Sampling for Seafood Species Labeling in Wholesale Seafood. https://www.fda.gov/downloads/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/Seafood/UCM419984.pdf Accessed 28 March 2019.

FDA. (2012b). Guidance for Industry: The Seafood List. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/guidance-industry-seafood-list Accessed 26 March 2019.

FDA. (2014). Seafood Species Substitution and Economic Fraud. https://www.fda.gov/food/seafood-guidance-documents-regulatory-information/seafood-species-substitution-and-economic-fraud Accessed 8 October 2017.

FDA. (2015). CPG Sec. 540.525 Decomposition and Histamine Raw, Frozen Tuna and Mahi-Mahi; Canned Tuna; and Related Species. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/cpg-sec-540525-decomposition-and-histamine-raw-frozen-tuna-and-mahi-mahi-canned-tuna-and-related Accessed 10 October 2017.

FDA. (2016). Guidance for Industry: Purchasing Reef Fish Species Associated with the Hazard of Ciguatera Fish Poisoning. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/guidance-industry-purchasing-reef-fish-species-associated-hazard-ciguatera-fish-poisoning Accessed 11 October 2017.

FDA. (2017). Federal Food, Drug, and Cosmetic Act (FD&C Act).

https://www.fda.gov/regulatory-information/laws-enforced-fda/federal-food-drug-and-cosmetic-act-fdc-act Accessed 8 October 2017.

FDA. (2018a). Regulatory Fish Encyclopedia. https://www.fda.gov/food/foodscienceresearch/rfe/ Accessed 18 December 2018.

FDA. (2018b). The Seafood List. https://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=seafoodlist Accessed 24 December 2018.

FishBase. (2018). FishBase. http://www.fishbase.org/search.php Accessed 1 August 2018.

Handy, S. M., Deeds, J. R., Ivanova, N. V., Hebert, P. D. N., Hanner, R. H., Ormos, A., . . . Yancy, H. F. (2011). A single-laboratory validated method for the generation of DNA barcodes for the identification of fish for regulatory compliance. *Journal of AOAC International*, *94*(1), 201-210.

Handy, S. M., Mueller, S., Jacob, S. M., Paul, S. Z., Garrett, S. D., & Deeds, J. R. (2017). Evaluation of the agilent technologies bioanalyzer-based DNA fish identification solution. *Food Control*, *73*B, 627-633.

Hebert, P. D. N., Cywinska, A., Ball, S. L., & deWaard, J. R. (2003). Biological identifications through DNA barcodes. *Proceedings: Biological Sciences*, *270*(1512), 313-321.

Hebert, P. D. N., Ratnasingham, S., & deWaard, J. R. (2003). Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. *Proceedings: Biological Sciences*, *270*, S96-S99.

Hebert, P. D. N., & Ratnasingham, S. (2007). Barcoding BOLD: The Barcode of Life

Data system (www.barcodinglife.org). *Molecular Ecology Notes, 7*(3), 355-364.

Hellberg, R., & Morrissey, M. (2011). Advances in DNA-based techniques for the detection of seafood species substitution on the commercial market. *Journal of Laboratory Automation, 16*(4), 308-321.

Hellberg, R., Pollack, S., & Hanner, R. (2016). Seafood species identification using DNA sequencing. In A. Naaum & R. Hanner (Eds.), *Seafood authenticity and traceability: A DNA-based perspective* (pp. 113-127). San Diego: Elsevier.

Hsieh, Y-H. P., Woodward, B. B., & Blanco, A. W. (1995). Species substitution of retail snapper fillets. *Journal of Food Quality*, *18*, 131-140.

Hu, Y., Huang, S. Y., Hanner, R., Levin, J., & Lu, X. (2018). Study of fish products in Metro Vancouver using DNA barcoding methods reveals fraudulent labeling. *Food Control*, *94*, 38-47.

Hulley, E. N., Tharmalingam, S., Zarnke, A., & Boreham, D. R. (2019). Development and validation of probe-based multiplex real-time PCR assays for the rapid and accurate detection of freshwater fish species. *PLoS ONE*, *14*(1), 1-20.

IUCN. (2019). The IUCN Red List of Threatened Species. https://www.iucn.org/resources/conservation-tools/iucn-red-list-threatened-species Accessed 2 January 2019.

Khaksar, R., Carlson, T., Schaffner, D. W., Ghorashi, M., Best, D., Jandhyala, S., . . . Amini, S. (2015). Unmasking seafood mislabeling in US markets: DNA barcoding as a unique technology for food authentication and quality control. *Food Control*, *56*, 71-76.

Lagasse, L. P., Love, D. C., & Smith, K. C. (2014). Country-of-Origin labeling prior to

and at the point of purchase: an exploration of the information environment in Baltimore City grocery stores. *Ecology of Food and Nutrition*, *53*(1), 58-80.

Luna, S. M., & Capuli, E. E. (2019). Gadus macrocephalus. https://www.fishbase.se/summary/Gadus-macrocephalus.html Accessed 4 April 2019.

Marko, P. B., Lee, S. C., Rice, A. M., Gramling, J. M., Fitzhenry, T. M., McAlister, J. S., . . . Moran, A. L. (2004). Mislabeling of a depleted reef fish. *Nature*, *430*, 309-310.

Marín, A., Fujimoto, T., & Arai, K. (2013). Rapid species identification of fresh and processed scallops by multiplex PCR. *Food Control*, *32*(2), 472-476.

Mitchell, J. K., & Hellberg, R. S. (2016). Use of the mitochondrial control region as a potential DNA mini-barcoding target for the identification of canned tuna species. *Food Analytical Methods*, *9*(10), 2711-2720.

Moore, M. M., Handy, S. M., Haney, C. J., Pires, G. S., Perry, L. L., Deeds, J. R., & Yancy, H. F. (2012). Updates to the FDA single laboratory validated method for DNA barcoding for the species identification of fish. *Journal of AOAC International*, *94*(1), 201-210.

Naaum, A., & Hanner, R. (2016). An introduction to DNA-based tools for seafood identification. In A. Naaum & R. Hanner (Eds.), *Seafood authenticity and traceability: a DNA-based perspective* (pp. 100-110). San Diego: Elsevier.

Nagalakshmi, K., Annam, P.K., Venkateshwarlu, G., Pathakota, G.-B., & Lakra, W. S. (2016). Mislabeling in Indian seafood: An investigation using DNA barcoding. *Food Control*, *59*, 196-200.

NOAA. (2015). Fisheries of the United States. https://www.st.nmfs.noaa.gov/Assets/commercial/fus/fus15/documents/FUS2015.pdf Accessed 25 September 2017.

NOAA. (2017). U.S. Aquaculture. https://www.fisheries.noaa.gov/national/aquaculture/us-aquaculture Accessed 25 September 2017.

NOAA. (2019). Atlantic Cod. https://www.fisheries.noaa.gov/species/atlantic-cod Accessed 1 January 2019.

Pollack, S. J., Kawalek, M. D., Williams-Hill, D. M., & Hellberg, R. S. (2018). Evaluation of DNA barcoding methodologies for the identification of fish species in cooked products. *Food Control*, *84*, 297-304.

Rahman, M., Heng, L. Y., Futra, D., Chiang, C. P., Rashid, Z. A., & Ling, T. L. (2017). A highly sensitive electrochemical DNA biosensor from acrylic-gold nano-composite for the determination of arowana fish gender. *Nanoscale Research Letters*, *12*(1), 484.

Shehata, H. R., Naaum, A. M., Garduno, R. A., & Hanner, R. (2018). DNA barcoding as a regulatory tool for seafood authentication in Canada. *Food Control*, *92*, 147-153.

Shokralla, S., Hellberg, R. S., Handy, S. M., King, I., & Hajibabaei, M. (2015). A DNA mini-barcoding system for authentication of processed fish products. *Scientific Reports*, *5*, Article number: 15894.

Stern, D. B., Castro Nallar, E., Rathod, J., & Crandall, K. A. (2017). DNA barcoding analysis of seafood accuracy in Washington, D.C. restaurants. *PeerJ*, e3234.

Tetzlaff, C., & Mäde, D. (2017). Development of a real-time PCR system for the detection of the potential allergen fish in food. *European Food Research and Technology*, *243*(5), 849-857.

Unicomb, L. E., Kirk, M., Yohannes, K., Dalton, C. B., & Halliday, L. (2002). An outbreak of gastrointestinal illness associated with the consumption of escolar fish. *Communicable diseases intelligence quarterly report*, *26*(3), 441-445.

USDA. (2017a). Country of Origin Labeling (COOL). https://www.ams.usda.gov/rules-regulations/cool Accessed 25 September 2017.

USDA. (2017b). Country of Origin Labeling (COOL) Frequently Asked Questions. https://www.ams.usda.gov/rules-regulations/cool/questions-answers-consumers Accessed 25 September 2017.

Wang, D., & Hsieh, Y.-H. P. (2016). The use of imported Pangasius fish in local restaurants. *Food Control*, *65*, 136-142.

Warner, K., Timme, W., Lowell, B., & Hirshfield, M. (2012). Widespread Seafood Fraud Found in Los Angeles. http://oceana.org/reports/widespread-seafood-fraud-found-los-angeles Accessed 1 September 2017.

Warner, K., Timme, W., Lowell, B., & Hirshfield, M. (2013). Oceana Study Reveals Seafood Fraud Nationwide. http://oceana.org/reports/oceana-study-reveals-seafood-fraud-nationwide Accessed 1 September 2017.

Wenne, R., Handschuh, L., Pocwierz-Kotus, A., Urbaniak, R., Formanowicz, P., Calkiewicz, J., . . . Wrobel, B. (2011). The application of microarray technology to the identification of Tc1-like element sequences in fish genomes. *Marine Biology Research*, *5*, 466-477.

Willette, D., Simmonds, S., Cheng, S., Esteves, S., Kane, T., Nuetzel, H., . . . Barber, P. (2017). Using DNA barcoding to track seafood mislabeling in Los Angeles restaurants. *Conservation Biology, 31*(5), 1076-1085.

Wong, E. H. K., & Hanner, R. H. (2008). DNA barcoding detects market substitution in North American seafood. *Food Research International, 41*(8), 828-837.

Yancy, H. F., Zemlak, T. S., Mason, J. A., Washington, J. D., Tenge, B. J., Nguyen, N. L. T., . . . Moore, M. M. (2008). Potential use of DNA barcodes in regulatory science: applications of the Regulatory Fish Encyclopedia. *Journal of Food Protection, 71*(1), 210-217.