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Authentication of Red Snapper (*Lutjanus campechanus*) Fillets Using a Combination of Real-time PCR and DNA Barcoding

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Comments

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Title: Authentication of Red Snapper (*Lutjanus campechanus*) Fillets using a Combination of Real-Time PCR and DNA Barcoding

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Abstract

Red snapper (*Lutjanus campechanus*) is a historically overfished and highly valued species that is commonly substituted with other fish, such as tilapia, rockfish, and other snapper species. The objective of this study was to assess the ability of real-time PCR to be used as a screening tool to rapidly test commercial fillets for the presence of red snapper, followed by species identification of negative samples with DNA barcoding. A total of 24 frozen, fresh, or thawed (previously frozen) fillets labeled as "red snapper" were tested with real-time PCR, along with 54 fillets from fish that are common substitutes for red snapper. Real-time PCR parameters were optimized to reduce cross-reactivity. All samples were also tested with DNA barcoding to confirm the identity of fish species. Among the 78 total samples, 3 were authenticated as red snapper with DNA barcoding and successfully detected with real-time PCR. An additional two samples were initially identified as red snapper with real-time PCR but confirmed negative with DNA barcoding, resulting in a false positive rate of 2.7%. Overall, 39.7% of all samples and 91.7% of "red snapper" samples were mislabeled. Red snapper was substituted with other snapper species, rockfish, sea bream, and mahi-mahi. These results illustrate the ability of real-time PCR to be used as a screening tool and the importance of species confirmation with DNA barcoding. Real-time PCR has the potential to be used as a rapid on-site screening tool for regulatory and industry officials to determine the authenticity of red snapper fillets.

Keywords: fish authentication; fraud; mislabeling; real-time PCR; red snapper; DNA barcoding

1. Introduction

Seafood fraud is a worldwide problem, in part due to limited monitoring and a complex global supply chain (Hellberg & Morrissey, 2011). According to the Food and Agriculture Organization of the United Nations (FAO), fish account for close to 20% of the average per capita intake of animal protein for over 3 billion people (FAO, 2018). Species substitution and mislabeling are common forms of seafood fraud committed throughout the supply chain. Traditionally, fish species are identified based on morphological characteristics, however, once processed into fillets, it is difficult to identify species that are similar in appearance (Pollack, Kawalek, Williams-Hill, & Hellberg, 2018; Shokralla, Hellberg, Handy, King, & Hajibabaei, 2015). A large-scale U.S. market survey reported mislabeling in one third of 1,247 seafood samples, with snappers and tunas having the highest individual mislabeling rates (Warner, Timme, Lowell, & Hirshfield, 2013). Seafood species mislabeling is often carried out for the purpose of economic deception; however, it can result in health risks to consumers (Pollack et al., 2018). For example, it can result in unanticipated exposure to allergens and/or toxins found in certain species of fish, such as tetrodotoxin, gempylotoxin, scrombrotoxin, or methylmercury (FDA, 2019a, 2019b).

One of the most commonly substituted fish is the red snapper (*Lutjanus campechanus*), with reported mislabeling rates of 73-100% (Khaksar et al., 2015; Marko et al., 2004; Spencer & Bruno, 2019; Warner et al., 2013; Willette et al., 2017; Wong & Hanner, 2008). The labeling of any fish other than *Lutjanus campechanus* as “red snapper” is considered misbranding and is in violation of the of the Federal Food, Drug, and Cosmetic Act (FDA, 1980). Red snapper are typically found in the Gulf of Mexico and along the nearby eastern coasts of the Americas (NOAA, 2019). Historic overfishing of red snapper combined with restrictive fishing regulations and population rebuilding plans have led to a limited supply of red snapper on the commercial market. Due to the large-scale popularity of red snapper combined with limited availability, this species is highly vulnerable to seafood substitution for economic gain. Common substitutions for red snapper include tilapia, rockfish, pollock, bream, flounder, bass, and other snapper species (Warner et al., 2013).

Currently, the FDA utilizes a DNA sequencing-based technique called DNA barcoding to monitor seafood species labeling (Handy et al., 2011). DNA barcoding can authenticate fish species based on a region of the cytochrome *c* oxidase subunit I (CO1) gene (Handy et al., 2011; Hebert, Cywinska, Ball, & Dewaard, 2003; Pollack et al., 2018). Full-barcoding uses a ~650 bp region of this gene and is very effective at identifying species from fresh or lightly processed samples (Cawthorn, Duncan, Kastern, Francis, & Hoffman, 2015; Christiansen, Fournier, Hellemans, & Volckaert, 2018; Willette et al., 2017). Mini-barcodes based on ~100-300 bp regions of CO1 have also been developed for use when full barcodes are not successful, for example with highly processed samples (Pollack et al., 2018; Shokralla et al., 2015).

While DNA barcoding has been demonstrated to be a highly effective technique for fish species identification, it is relatively costly and can require multiple days of laboratory analysis. On the other hand, real-time polymerase chain reaction (PCR) integrates DNA amplification and fluorescence techniques to identify a sample without the need for gel electrophoresis or DNA sequencing (Hellberg & Morrissey, 2011). Real-time PCR assays have the potential to authenticate seafood products on-site, thereby eliminating excess costs and time (Naaum, Hellberg, Okuma, & Hanner, 2019). Real-time PCR has been reported to be a highly sensitive, specific and rapid technique for the identification of fish species in previous studies (reviewed in Applewhite, Larkin, & Naaum, 2016; Hellberg & Morrissey, 2011). Despite the extensive research conducted in this area, there are currently no real-time PCR assays available for the detection of red snapper in commercial fish products. A real-time PCR assay was previously developed for the detection of red snapper and two other species in fish eggs from the Gulf of Mexico for fisheries management applications (Bayha, Graham, & Hernandez Jr, 2008). The assay was found to be a reliable means for the detection of red snapper based on testing with over 60 samples of red snapper fin clips and eggs, as well as numerous samples from other target and non-target species. However, this assay has yet to be tested for its ability to detect red snapper species substitution among commercial fish fillets.

The objective of this study was to assess the ability of real-time PCR to be used as a screening tool to rapidly test commercial fillets for the presence of red snapper, followed by species identification of negative samples with DNA barcoding. Because the real-time PCR assay developed by Bayha et al. (2018) was already verified as a reliable method for the identification of red snapper, the main goal of the current study was to apply this assay to commercial fillets of red snapper and common substitute species. The results of this study are meant to present a rapid method that can be used on-site to authenticate fish products and ensure proper labeling of red snapper fillets in the seafood market.

2. Materials and Methods

2.1 Sample collection

A total of 78 frozen, fresh, or thawed (previously frozen) fish fillets were purchased from 18 online retailers and 4 grocery stores in Orange County, CA, USA. A total of 24 fillets labeled as “red snapper” were collected and 54 fillets of common substitute species from the following categories of fish: bass, catfish, cod, flounder, halibut, mahi-mahi, perch, pollock, rockfish, other snapper species, sole, tilapia, and tilefish. The place of purchase, price per pound, and stated identity of all samples were recorded, and each sample was catalogued and assigned a sample identification number. Fresh or thawed fish were processed for DNA extraction immediately upon arrival at the laboratory while frozen samples were thawed overnight at 4°C before DNA extraction.

2.2 DNA extraction

DNA extraction was carried out with the DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA, USA), Spin-Column protocol, with modifications described in Handy et al. (2011). Tissue samples (~10 mg) were aseptically removed from the interior of each fillet using a sterile scalpel and placed in a sterile 1.5 mL microcentrifuge tube. Samples were lysed at 56°C for 2 h with a ThermoMixer C (Eppendorf, Hamburg, Germany) set at 300 rpm. DNA was eluted using 100 µl of pre-heated (37°C) AE buffer (Qiagen). Each set of DNA extractions included a reagent blank negative control with no added sample. A Biophotometer Plus (Eppendorf) was used to quantify the DNA in each sample. DNA extracts were stored at 4°C until further analysis.

2.3 Real-time PCR screening for red snapper

All DNA extracts underwent real-time PCR using the Rotor-Gene Q (Qiagen) with the reaction mixture described in Bayha et al. (2008): 10.0 µl of iQ™ supermix (2x) (Bio-Rad Laboratories, Hercules, CA, USA), 5.00 µl of molecular grade water, 1.80 µl of 10 µM DMT-172 primer (Table 1), 0.80 µl of 10 µM DMT-175 primer (Table 1), 0.40 µl of 10 µM DMT-180 locked nucleic acid (LNA) probe (Table 1), and 2.00 µl of template DNA (5-30 ng/µl). The primers were synthesized by Integrated DNA Technologies (Coralville, IA, USA) and the probe was synthesized by Sigma-Aldrich (St. Louis, MO, USA). A no-template control (NTC) with molecular-grade sterile water in place of DNA was included alongside each set of reactions.

Additionally, each real-time PCR run contained red snapper positive control DNA at the following concentrations: 12 ng/µl, 1.2 ng/µl, and 0.12 ng/µl. The red snapper positive control DNA was extracted from one of the samples (R58) collected for this study and was verified to be red snapper with DNA barcoding (described below). The cycling conditions for real-time PCR were: 95°C for 8 min; 38 cycles of 95°C for 15 s and 62°C for 35 s. Results were analyzed with the Rotor-Gene Q software (Qiagen) using the following parameters: threshold = 0.09, slope correct, and ignore first 5 cycles. Due to the observance of cross-species amplification after 30 cycles, samples were only considered positive for red snapper if a Ct value less than 30 was obtained. In instances where the real-time PCR identification was not consistent with the results of DNA barcoding (described below), the samples (R71 and R78) underwent repeat DNA extraction and real-time PCR.

2.4 DNA barcoding for species confirmation

Each DNA extract underwent full DNA barcoding targeting a 655 bp region of CO1 as described in Moore et al. (2012). All primers used in this study are shown in Table 1. PCR amplification was carried out with the following reaction mixture: one half of an OmniMix® HS PCR Master Mix bead (Cepheid, Sunnyvale, CA, USA), 12.5 µl of 10% trehalose, 8.00 µl molecular grade water, 0.25 µl of 10 µM forward primer (FISHCO1LBC_ts), 0.25 µl of 10 µM reverse primer (FISHCO1HBC_ts), and 2.00 µl of template DNA (5-30 ng/µl). The cycling conditions for full-barcoding were: 94°C for 2 min; 35 cycles of 94°C for 30 s, 55°C for 40 s, and 72°C for 1 min; and a final extension step at 72°C for 10 min. Samples that failed to be identified with full barcoding (n = 15) underwent mini-barcoding with the SH-E mini-barcode primers targeting a 226 bp region of CO1 (Table 2) (Shokralla et al., 2015). Each mini-barcoding reaction contained: one half of an OmniMix® HS Lyophilized PCR Master Mix bead, 22.0 µl molecular grade water, 0.50 µl of 10 µM forward primer (Mini_SH-E_F), 0.50 µl of 10 µM reverse primer (Mini_SH-E_R) and 2.00 µl of template DNA. The cycling conditions for mini-barcoding were: 95°C for 5 min; 35 cycles of 94°C for 40 s, 46°C for 1 min, and 72°C for 30 s; and a final extension of 72°C for 5 min. All primers were synthesized by Integrated DNA Technologies. A no-template control (NTC) with molecular-grade sterile water in place of DNA was included alongside each set of reactions. PCR was carried out using a Mastercycler nexus Gradient Thermal Cycler (Eppendorf).

PCR products were confirmed using 2.0% agarose E-Gels (Invitrogen, Carlsbad, CA, USA) run on an E-Gel iBase (Invitrogen). Each well was loaded with 16 µl of sterile water and 4 µl of PCR product (Hellberg, Kawalek, Van, Shen, & Williams-Hill, 2014). Gels were run for 15 min and the results were captured using FOTO/Analyst Express (Fotodyne, Hartland, WI, USA) and Transilluminator FBDLT-88 (Fisher Scientific, Waltham, MA, USA) combined with FOTO/Analyst PCImage (version 5.0.0.0, Fotodyne). All samples with confirmed PCR products underwent clean-up with ExoSAP-IT (Affymetrix, Santa Clara, CA, USA), according to the manufacturer's instructions. The purified PCR products were then shipped to GenScript (Piscataway, NJ, USA) for DNA sequencing with M13 primers.

Sequence assembly and editing were completed using Geneious R7 (Biomatters, Ltd., Auckland, New Zealand). The resulting sequences were queried through the Barcode of Life Database (BOLD) Animal Identification Request Engine (http://www.boldsystems.org/), Species Level Barcodes, and GenBank with the Nucleotide Basic Local Alignment Search Tool (BLASTn; http://blast.ncbi.nlm.nih.gov/Blast.cgi). The top fish species match with at least 98% genetic similarity for each sample was recorded as the identified species.

3. Results and Discussion

3.1 Real-time PCR screening for red snapper

Among the 78 fish fillets analyzed in this study, 5 initially tested positive for red snapper with real-time PCR (Table 2). Three of these samples were authenticated as red snapper with DNA barcoding and two were determined to be false positives. Among the red snapper fillets authenticated with DNA barcoding, two were labeled as "red snapper" (R70 and R51) and one was labeled as "yellowtail red snapper" (R58). One of the false positive samples (R78) was labeled as "red snapper" but identified as mahi-mahi (*Coryphaena hippurus*) with DNA barcoding. Due to the disagreement in species assignment for the mahi-mahi sample, it underwent repeat DNA extraction and real-time PCR analysis. However, it continued to show a positive result (Ct = 20.41) for red snapper with real-time PCR. This result was likely due to cross-contamination of the mahi-mahi fillet with red snapper DNA during shipping. The mahi-mahi fillet was delivered in the same packaging as an authenticated red snapper sample, with no physical separation between the two fillets. The two fillets arrived in a partially thawed state and there was likely some exchange of liquid between the authentic red snapper fillet and the mahi-mahi fillet during shipping. Although sampling was conducted on the interior of each fillet, real-time PCR is a highly sensitive assay and likely detected red snapper in the mahi-mahi fillet because of the close contact between the two samples. The other sample (R71) that was determined to be a false positive was identified as blue tilefish (*Lopholatilus chamaeleonticeps*) with DNA barcoding but had a Ct value of 27.86 with real-time PCR. After repeating the DNA extraction and real-time PCR analysis for the sample, the sample tested negative for red snapper. These results indicate cross-reactivity of the red snapper assay with the blue tilefish sample. Of note, the three authenticated red snapper samples showed Ct values of 16.48-18.88. Lowering the Ct value required for a positive detection to <25 would help reduce the chance of false positives in future testing.

The remaining 73 samples in this study tested negative for red snapper with real-time PCR, in agreement with the results of DNA barcoding. Of note, a sample (R62) labeled "red snapper" was identified as Pacific snapper (*Lutjanus peru*) (100% genetic similarity) with DNA barcoding but had a secondary species match with *L. campechanus* in BOLD (98.77% genetic similarity). Although the *Lutjanus* species are genetically similar, the species-specific primers and probes in real-time PCR did not amplify the DNA, thus confirming that the species was mislabeled. Overall, the real-time PCR assay identified 100% (n=3) of true red snapper samples with only 2 false positives out of the 75 negative samples (2.7%). Because real-time PCR was able to correctly identify all authentic red snapper samples, this assay could be used as a rapid screening method to verify correctly labeled fillets of red snapper within ~5 h. Only samples that test negative for red snapper with real-time PCR would need to be tested with DNA barcoding. The use of real-time PCR as a screening method would limit the need for DNA barcoding, which typically takes at least 1-2 days for species identification and is generally more costly than real-time PCR (Naaum et al., 2019).

3.2 DNA barcoding for species confirmation

All 78 fish fillets were identified with full or mini-barcoding (Table 2), with > 99% genetic similarity to at least one fish species. Full barcoding enabled identification of 63 samples, including all three red snapper fillets (R51, R58, R70). The remaining 15 samples were identified with mini-barcoding as snapper species (*Lutjanus* spp., *Ocyurus chrysurus*, *Pristipomoides typus*, *Rhomboplites aurorubens*; n=5), seabream (*Pagrus* spp.; n=3), catfish (*Ictalurus punctatus*; n=2), rockfish (*Sebastes* spp.; n=1), thornyhead (*Sebastolobus* spp.; n=1), Atlantic halibut (*Hippoglossus hippoglossus*; n=1), pollock (*Pollachius virens*; n=1), and mahi-mahi (*Coryphaena hippurus*; n=1). Interestingly, 8 out of the 24 samples labeled as "red snapper" failed sequencing with the full-barcode primers but were identified as species other than red snapper with mini-barcoding (Table 2). The primers used for mini-barcoding and full barcoding have different sequences (Table 1), meaning that fish that are unable to be amplified with full barcoding may be successfully amplified with mini-barcoding. Similarly, a previous study on fresh and thawed fish fillets reported the ability to identify samples with mini-barcoding when full barcoding failed (Liou, Banda, Isaacs, & Hellberg, et al., 2020).

When the genetic identification results of BOLD and GenBank were combined, a total of 46 samples were identified to the species level and 26 samples were identified to the genus level. The samples identified to the genus level showed equivalent genetic similarity to more than one species from the same genus in BOLD and/or GenBank [*Oreochromis* spp. (n=10), *Lutjanus* spp. (n=6), *Sebastes* spp. (n=4), *Pagrus* spp. (n=4), *Sebastolobus* spp. (n=1), *Cynoscion* spp. (n=1)]. An additional 6 samples showed equivalent genetic similarity to more than one species from multiple genera [*Boreogadus/Gadus* spp. (n=5), *Pangasianodon/Pangasius* spp. (n=1)]. All 78 of the samples had top genetic matches in both BOLD and GenBank, except for one sample (R74) that did not show any genetic matches in BOLD and was identified based solely on GenBank. This sample was identified as Cortez halibut (*Paralichthys aestuarius*) with 99.69% genetic similarity. There were no entries for this species in BOLD, which explains why a match could not be obtained with this database.

The majority of samples (n = 62) showed the exact same top species match in both BOLD and GenBank (Table 2). However, there were a few categories of fish, such as cod, seabream, and snappers, that showed slight differences in the top species identifications between the two databases. For example, five samples labeled as cod (R08, R11, R17, R28, and R34) showed an equivalent top match to three species in BOLD (*Gadus macrocephalus, Boreogadus saida,* and *Gadus ogac*) but only two of these species in GenBank (*Gadus macrocephalus* and *Gadus ogac*). However, the sequences for *Boreogadus saida* were listed as "Early Release" and likely were not deposited in GenBank. With regards to snapper species, there were three samples (R51, R58, R70) labeled as red snapper that showed equivalent top genetic matches to both *Lutjanus campechanus* and *Lutjanus purpureus* in BOLD, but only a single top genetic match to *Lutjanus campechanus* in GenBank. The *Lutjanus purpureus* records in BOLD had been mined from GenBank, but likely due to differences in the two search engines, they did not appear as a top match in GenBank. An additional sample (R49) had equivalent top genetic matches to both *Lutjanus erythropterus* and *Lutjanus malabaricus* in BOLD, but only a single top genetic match to *Lutjanus erythropterus* in GenBank. The BOLD record for *Lutjanus malabaricus* was listed as a private entry and may not have been deposited in GenBank.

3.3 Species mislabeling in fish fillets

Overall, 31/78 (39.7%) samples were determined to be mislabeled due to either species substitution or use of unacceptable market names (Table 3). Of the 24 samples analyzed that were marketed as "red snapper," DNA barcoding revealed that only 2 were compliant with species labeling (8.3%). The most common substitutes for red snapper in this study were other snapper species, including Malabar snapper (*Lutjanus malabaricus*; n=5), vermilion snapper (*Rhomboplites aurorubens*; n=2), Pacific snapper (n=1), yellowtail snapper (*Ocyurus chrysurus*; n=1), and other *Lutjanus* spp. (n=3). These results are consistent with previous literature, which has reported the substitution of red snapper with other snappers, such as Malabar snapper (Hsieh, Woodward, & Blanco, 1995; Spencer & Bruno 2019), Pacific snapper (Warner et al., 2013), vermilion snapper (Hsieh et al., 1995; Marko et al., 2004; Shehata, Naaum, Garduño, & Hanner, 2018; Spencer & Bruno 2019; Warner et al., 2013), and yellowtail snapper (Hsieh et al., 1995; Spencer & Bruno 2019; Warner et al., 2013). Interestingly, two samples advertised as "Red Snapper Fillet" (R27 and R77) purchased over 4 months apart from the same online retailer were consistently identified as Malabar snapper.

In addition to other snapper species, red snapper was found to be substituted with sea bream species (*Pagrus* spp.), specifically madai (*Pagrus major*) and squirefish (*Pagrus auratus*), in four samples (R41, R42, R43, R46) purchased from two different Japanese grocery stores. These results are in agreement with previous studies (Khaksar et al., 2015; Liou et al., 2020; Warner et al., 2013; Willette et al., 2017). There may be confusion over the acceptable market name of these species as madai is considered genuine snapper in sushi culture (Hu, Huang, Hanner, Levin, & Lu, 2018).

Rockfish species [*Sebastes flavidus* (n=1) or *Sebastes brevispinis* (n=1)] continued to be substitutes for red snapper in this study, in agreement with numerous other studies (Liou et al., 2020; Shehata et al., 2018; Warner et al., 2013; Willette et al., 2017; Wong & Hanner, 2008). These two samples were both labeled as "Red Snapper Fillet" (R39 and R75) and were purchased from the same online retailer over 4 months apart, indicating that this retailer is consistently mislabeling its product. As discussed in previous studies, the mislabeling of rockfish as red snapper may be due in part to confusion in naming: in the state of California, some rockfish species (including *S. flavidus*) may be sold under the name "Pacific red snapper" (14 CCR § 103). However, the samples identified as *S. flavidus* and *S. brevispinis* in this study were labeled specifically as "red snapper" and not "Pacific red snapper." Of note, "Pacific red snapper" is not an acceptable market name for any species according to the FDA Seafood List

(FDA, 2020a). Unique to this study, one sample of red snapper was found to be substituted with mahi-mahi (*Coryphaena hippurus*; R78). Interestingly, the online company that sold the substituted sample also sells mahi-mahi fillets. The fillet identified as mahi-mahi was shipped in the same packaging as an authenticated red snapper fillet but was different in appearance, with a darker pink color on the surface of the fillet (Figure S1). The substitution of red snapper with mahi-mahi presents a potential health risk due to the association of mahi-mahi with scombrotoxin (histamine) (FDA, 2019b).

Three of the 24 samples labeled as "red snapper" were determined to have unacceptable market names because they combined the names of multiple species. One sample was labeled as "Yellowtail Red Snapper Fillet" (R58), which combines the names of two separate species: yellowtail snapper and red snapper. The sample was identified to be red snapper by both DNA barcoding and real-time PCR. Another sample was labeled as "Local Red Snapper/Rockfish" (R52), which combines the names of red snapper and rockfish (*Sebastes* spp.). This sample was identified as Pacific ocean perch (*Sebastes alutus*) with DNA barcoding. An additional sample (R64) was found to have conflicting market names: it was advertised online as "Wild Caught Red Snapper Filets" but the package label declared "Snapper Fillet (*Pristipomoides* spp.)" This sample was identified with DNA barcoding as sharptooth jobfish (*Pristipomoides typus*), which can acceptably be marketed as "snapper" but not "red snapper." Of note, the company that sold the mislabeled fillet does not list any snappers other than red snapper for sale on its website. Substitution of red snapper with sharptooth jobfish was previously reported by Wong and Hanner (2008) and Warner et al. (2013).

Among the 54 samples that did not include "red snapper" as part of the market name, 9 (16.7%) were considered mislabeled, including samples labeled as halibut (n=3), cod (n=2), akauo (n=1), bass (n=1), flounder/halibut (n=1) and flounder (n=1). One of the mislabeled cod samples (R05) was labeled as "Pacific cod" (*Gadus macrocephalus*) but identified as Atlantic cod (*Gadus morhua*). Another cod sample (R11) was identified as Pacific cod but labeled as "true cod." True cod is considered to be a vernacular name for Pacific cod and is not an acceptable market name according to the FDA Seafood List. One sample was labeled "Akauo kirimi wild Alaska" (R44); however, akauo is not an acceptable market name for any species according to the FDA Seafood List. This sample was identified as thornyhead (*Sebastolobus* spp.) with DNA barcoding.

Halibut and flounder are often interchangeable in their vernacular names; however, the term "halibut" may legally only be used to refer to Atlantic halibut (*Hippoglossus hippoglossus*) or Pacific halibut (*Hippoglossus stenolepis*) (21 CFR 102.57). Therefore, samples labeled with the term halibut that were not identified as one of these two species were determined to be mislabeled on the basis of species substitution. One of the mislabeled halibut samples (R45) was labeled as "Hirame Halibut"; however, this is not an acceptable market name for any species on the FDA Seafood List. According to FDA guidance, hirame is a commonly used Japanese vernacular name for fluke or flounder (FDA, 2020b), which is consistent with the DNA barcoding identification of olive flounder (*Paralichthys olivaceus*) for this sample. The other two mislabeled halibut samples (R55 and R74) both contained the term "local halibut" to describe other flounder species. Sample R55 was identified as California flounder (*Paralichthys californicus*) and R74 was identified as Cortez halibut. According to the FDA Seafood List, "California halibut" is a vernacular name for California flounder, which can cause confusion in terms of labeling as vernacular names are not acceptable market names. Although the common name of Cortez halibut contains the term "halibut," this fish cannot legally be marketed as a halibut (21 CFR 102.57). An additional sample (R47) was marketed on a website as "Proton-Frozen Local Halibut Fillet" but the label on the vacuum package described the fish as "Frozen Flounder Fillet." This sample was identified as speckled flounder (*Paralichthys woolmani*) and was considered to be mislabeled due to the conflicting market names between the website and the label. Of note, the company does not list any products for sale under the name of "flounder" on its website.

A sample labeled as "Flounder Fillet" (R48) was found to be substituted with Pangasius (*Pangasianodon hypophthalmus* or *Pangasius* spp.). Economically motivated substitution seems likely with this sample, which was purchased for US $41.86/kg. On the other hand, Pangasius fish are relatively low in price, with an average retail cost of US $9.91/kg reported in a previous study (Liou et al., 2020). Sample R53 was labeled as "White Sea Bass" but identified as weakfish (*Cynoscion* spp.). The FDA Seafood List states that white seabass is the common name for *Atractoscian nobilis* so this sample was considered mislabeled on the basis of species substitution.

4. Conclusions

This study highlights the potential use of real-time PCR to facilitate the detection of red snapper species substitution within the seafood industry. This method was found to be suitable for use as a rapid screening tool when testing for the presence of red snapper fillets. All three red snapper samples authenticated with DNA barcoding were successfully detected with real-time PCR. Real-time PCR had a low rate of false-positive detections (2.7%), which were confirmed negative with DNA barcoding. This method enables identification of red snappers within about 5 h of sample collection. If needed, sample results can then be verified with DNA barcoding. A combination of DNA full barcoding and mini-barcoding enabled the identification of species in all 78 fish fillets tested in this study. Species mislabeling among red snapper and common substitute species was observed in 39.7% of all samples and 91.7% of samples labeled as "red snapper." The high mislabeling rate unveiled in this study and in previous research emphasizes the need for increased outreach and monitoring of seafood mislabeling, including the use of rapid identification techniques. Future research should be conducted to examine the feasibility of using this assay for on-site testing of red snapper fillets.

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- 78 red snapper and common substitute fillets were collected from retailers
- Real-time PCR correctly identified all authentic red snapper samples
- DNA barcoding confirmed the results of real-time PCR for 97.4% of samples
- Real-time PCR showed a low false positive rate of 2.7%
- 91.7% of samples sold as “red snapper” were mislabeled

Table 1. Primers and probes used in this study

PCR assay	Target region	Primer/probe name	Function	Sequence (3'-5')[a]	Reference
DNA full barcoding	CO1	FISHCOILBC_ts	Forward primer	CACGACGTTGTAAAACGACTCAACY AATCAYAAAGATATYGGCAC	Handy et al. (2011); Moore et al. (2012)
		FISHCOILBC_ts	Reverse primer	GGATAACAATTTCACACAGGACTTC YGGGTGRCCRAARAATCA	
DNA mini barcoding	CO1	Mini_SH-E	Forward primer	CACGACGTTGTAAAACGACACYAAI CAYAAAGAYATIGGCAC	Shokralla et al. (2015)
		Mini_SH-E	Reverse primer	GGATAACAATTTCACACAGGCTTAT RTTRTTTATICGIGGRAAIGC	
Real-time PCR for red snapper	Cytochrome *b*	DMT-172	Forward primer	GGATTAGCCATCCGTAATTTACA	Bayha et al. (2008)
		DMT-175	Reverse primer	TACAYTACACCTCCGACATCA	
		DMT-180	LNA Taqman probe	FAM-tcTcAtcAgtCgccca-BHQ1[b]	

[a]Underlined portions of primer sequences indicate M13 tails; Taqman LNA probe bases are in capital letters and normal bases are in small letters

[b]FAM was used in the current study in place of TET

Table 2. Results of sample testing with real-time PCR and DNA barcoding. DNA barcoding identifications are based on the results of full barcoding unless otherwise noted. Both Ct values are shown for samples that underwent a second real-time PCR.

Sample	Category	Source	Product name as advertised	Expected species	DNA barcoding identification		Real-time PCR results	
					BOLD	GenBank	Red snapper detected	Ct
R01	Catfish	Grocery Store A	Catfish Fillet	*Ictalurus* spp.	*Ictalurus punctatus*[a]	*Ictalurus punctatus*[a]	-	-
R02	Tilapia	Grocery Store A	Tilapia Fillet	*Oreochromis* spp.	*Oreochromis niloticus, Oreochromis aureus*	*Oreochromis niloticus, Oreochromis aureus*	-	-
R03	Sole	Grocery Store A	Dover Sole Fillet	*Microstomus pacificus*	*Microstomus pacificus*	*Microstomus pacificus*	-	-
R04	Rockfish	Grocery Store A	Fresh Wild Caught Rockfish Fillets	*Sebastes* spp.	*Sebastes proriger*	*Sebastes proriger*	-	-
R05	Cod, Pacific	Grocery Store A	Fresh Wild Caught Pacific Cod Fillets	*Gadus macrocephalus*	*Gadus morhua*	*Gadus morhua*	-	-
R06	Tilapia	Grocery Store A	Tilapia Fillet	*Oreochromis* spp.	*Oreochromis* spp.	*Oreochromis* spp.	-	-
R07	Rockfish	Grocery Store A	Fresh Wild Caught Rockfish Fillets	*Sebastes* spp.	*Sebastes* spp.	*Sebastes* spp.	-	-
R08	Cod, Pacific	Grocery Store A	Fresh Wild Caught Pacific Cod Fillets	*Gadus macrocephalus*	*Gadus macrocephalus, Boreogadus saida, Gadus ogac*	*Gadus macrocephalus, Gadus ogac*	-	-
R09	Sole	Grocery Store A	Pacific Sole Fllt Frsh (T/W)	*Microstomus pacificus*	*Microstomus pacificus*	*Microstomus pacificus*	-	-
R10	Catfish	Grocery Store A	Catfish Fillet Fresh – Farm Raised	*Ictalurus punctatus*	*Ictalurus punctatus*	*Ictalurus punctatus*	-	-
R11	Cod, Pacific	Grocery Store A	True Cod Fillet Fresh	*Gadus macrocephalus*	*Gadus macrocephalus, Boreogadus saida, Gadus ogac*	*Gadus macrocephalus, Gadus ogac*	-	-

R12	Rockfish	Grocery Store A	Pacific Rockfish Fillet Fresh Wild Caught	*Sebastes* spp.	*Sebastes entomelas*	*Sebastes entomelas*	-	-
R13	Sole	Grocery Store A	Sole Fillet Pacific Fresh Wild Caught	*Microstomus pacificus*	*Microstomus pacificus*	*Microstomus pacificus*	-	-
R14	Tilapia	Grocery Store A	Tilapia Fillet Fresh	*Oreochromis* spp.	*Oreochromis* spp.	*Oreochromis* spp.	-	-
R15	Tilapia	Grocery Store A	Tilapia Fillet Fresh	*Oreochromis* spp.	*Oreochromis* spp.	*Oreochromis* spp.	-	-
R16	Tilapia	Grocery Store B	Fresh Tilapia Fillet (farmed)	*Oreochromis* spp.	*Oreochromis niloticus, Oreochromis aureus*	*Oreochromis niloticus, Oreochromis aureus*	-	-
R17	Cod, Pacific	Grocery Store B	Alaska Cod Fillet Previously Frozen (wild)	*Gadus macrocephalus*	*Gadus macrocephalus, Boreogadus saida, Gadus ogac*	*Gadus macrocephalus, Gadus ogac*	-	-
R18	Tilapia	Online Retailer 1	Tilapia (farmed)	*Oreochromis* spp.	*Oreochromis* spp.	*Oreochromis* spp.	-	-
R19	Tilapia	Online Retailer 1	Tilapia (farmed)	*Oreochromis* spp.	*Oreochromis* spp.	*Oreochromis* spp.	-	-
R20	Red snapper	Online Retailer 1	Red Snapper (Sashimi grade)	*Lutjanus campechanus*	*Lutjanus malabaricus*	*Lutjanus malabaricus*	-	-
R21	Red snapper	Online Retailer 1	Red Snapper (Sashimi grade)	*Lutjanus campechanus*	*Lutjanus malabaricus*	*Lutjanus malabaricus*	-	-
R22	Mahi-mahi	Online Retailer 1	Mahi-mahi	*Coryphaena hippurus*	*Coryphaena hippurus*	*Coryphaena hippurus*	-	-
R23	Mahi-mahi	Online Retailer 1	Mahi-mahi	*Coryphaena hippurus*	*Coryphaena hippurus*[a]	*Coryphaena hippurus*[a]	-	-
R24	Catfish	Online Retailer 1	Guidry's Catfish Fillets	*Ictalurus* spp.	*Ictalurus punctatus*[a]	*Ictalurus punctatus*[a]	-	-
R25	Red snapper	Online Retailer 2	Red Snapper	*Lutjanus campechanus*	*Rhomboplites aurorubens*	*Rhomboplites aurorubens*	-	-
R26	Catfish	Online Retailer 2	Fresh Catfish (farmed)	*Ictalurus punctatus*	*Ictalurus punctatus*	*Ictalurus punctatus*	-	-
R27	Red snapper	Online Retailer 3	Red Snapper Fillet	*Lutjanus campechanus*	*Lutjanus malabaricus*	*Lutjanus malabaricus*	-	-
R28	Cod, unspecified	Online Retailer 3	Cod Fillet	*Gadus* spp.	*Gadus macrocephalus, Boreogadus saida, Gadus ogac*	*Gadus macrocephalus, Gadus ogac*	-	-

R29	Tilapia	Online Retailer 3	Tilapia Fillet	*Oreochromis* spp.	*Oreochromis* spp.	*Oreochromis* spp.	-	-
R30	Catfish	Online Retailer 3	Catfish Fillet	*Ictalurus* spp.	*Ictalurus punctatus*	*Ictalurus punctatus*	-	-
R31	Catfish	Online Retailer 4	Catfish Fillet	*Ictalurus* spp.	*Ictalurus punctatus*	*Ictalurus punctatus*	-	-
R32	Perch	Online Retailer 4	Ocean Perch Fillet	*Sebastes alutus*	*Sebastes* spp.	*Sebastes* spp.	-	-
R33	Tilefish	Online Retailer 4	Tilefish Fillet	*Lopholatilus chamaeleonticeps*	*Lopholatilus chamaeleonticeps*	*Lopholatilus chamaeleonticeps*	-	-
R34	Cod, Atlantic	Online Retailer 5	Cod - North Atlantic	*Gadus morhua*	*Gadus macrocephalus, Boreogadus saida, Gadus ogac*	*Gadus macrocephalus, Gadus ogac*	-	-
R35	Pollock	Online Retailer 5	Pollock - North Atlantic	*Pollachius virens*	*Pollachius virens*	*Pollachius virens*	-	-
R36	Sole	Online Retailer 5	Sole - North Atlantic	*Solea solea*	*Glyptocephalus cynoglossus*	*Glyptocephalus cynoglossus*	-	-
R37	Catfish	Online Retailer 6	Catfish Fillet Fresh	*Ictalurus* spp.	*Ictalurus punctatus*	*Ictalurus punctatus*	-	-
R38	Mahi-mahi	Online Retailer 6	Mahi-mahi Wild Frozen	*Coryphaena hippurus*	*Coryphaena hippurus*	*Coryphaena hippurus*	-	-
R39	Red snapper	Online Retailer 6	Red Snapper Fillet Wild Fresh	*Lutjanus campechanus*	*Sebastes flavidus*	*Sebastes flavidus*	-	-
R40	Tilapia	Online Retailer 6	Tilapia Fillet Fresh	*Oreochromis* spp.	*Oreochromis niloticus, Oreochromis mossambicus*	*Oreochromis* spp.	-	-
R41	Red snapper	Grocery Store C	Fresh Red Snapper Sashimi Farmed	*Lutjanus campechanus*	*Pagrus major, Pagrus auratus*[a]	*Pagrus major, Pagrus auratus*[a]	-	-
R42	Red snapper	Grocery Store D	Red Snapper from Japan	*Lutjanus campechanus*	*Pagrus major, Pagrus auratus*[a]	*Pagrus major, Pagrus auratus*[a]	-	-
R43	Red snapper	Grocery Store D	Red Snapper Wild New Zealand	*Lutjanus campechanus*	*Pagrus major, Pagrus auratus*	*Pagrus auratus*	-	-
R44	Perch	Grocery Store D	Akauo kirimi wild Alaska USA	Numerous possible species	*Sebastolobus alascanus, Sebastolobus altivelis*[a]	*Sebastolobus alascanus, Sebastolobus altivelis*[a]	-	-

R45	Halibut	Grocery Store D	Hirame Halibut Sashimi Live From Korea	*Hippoglossus hippoglossus* or *Hippoglossus stenolepis*	*Paralichthys olivaceus*	*Paralichthys olivaceus*	-	-
R46	Red snapper	Grocery Store C	Premium Red Snapper Japan Wild	*Lutjanus campechanus*	*Pagrus major*[a]	*Pagrus major, Pagrus auratus*	-	-
R47	Flounder/Halibut	Online Retailer 7	Package: Frozen Flounder Fillet Website: Proton-Frozen Local Halibut Fillet	*Paralichthys* spp.	*Paralichthys woolmani*[b]	*Paralichthys woolmani*[b]	-	-
R48	Flounder	Online Retailer 8	Flounder Fillet	*Paralichthys* spp.	*Pangasianodon hypophthalmus, Pangasius djambal*	*Pangasianodon hypophthalmus, Pangasius bocourti*	-	-
R49	Red snapper	Online Retailer 8	Red Snapper Fillet	*Lutjanus campechanus*	*Lutjanus erythropterus, Lutjanus malabaricus*	*Lutjanus erythropterus*	-	-
R50	Bass	Online Retailer 7	White Sea bass Fillet	*Atractoscion nobilis*	*Atractoscion nobilis*	*Atractoscion nobilis*	-	-
R51	Red snapper	Online Retailer 9	Fresh Red Snapper	*Lutjanus campechanus*	*Lutjanus campechanus, Lutjanus purpureus*	*Lutjanus campechanus*	+	18.71
R52	Red snapper/rockfish	Online Retailer 10	Local Red Snapper/Rockfish	*Sebastes* spp.	*Sebastes alutus*	*Sebastes alutus*	-	-
R53	Bass	Online Retailer 10	Santa Barbara White Sea Bass	*Atractoscion nobilis*	*Cynoscion xanthulus*[b]	*Cynoscion acoupa*	-	-
R54	Mahi-mahi	Online Retailer 10	Mahi-mahi	*Coryphaena hippurus*	*Coryphaena hippurus*	*Coryphaena hippurus*	-	-
R55	Halibut	Online Retailer 10	Local Halibut	*Hippoglossus hippoglossus* or *Hippoglossus stenolepis*	*Paralichthys californicus*	*Paralichthys californicus*	-	-
R56	Perch	Online Retailer 11	Ocean Perch Fillet	*Sebastes alutus*	*Sebastes* spp.	*Sebastes* spp.	-	-
R57	Cod, Atlantic	Online Retailer 11	Atlantic Cod Fillet	*Gadus morhua*	*Gadus morhua*	*Gadus morhua*	-	-

R58	Red snapper/ yellowtail snapper	Online Retailer 11	Yellowtail Red Snapper Fillet	*Ocyurus chrysurus* or *Lutjanus campechanus*	*Lutjanus campechanus, Lutjanus purpureus*	*Lutjanus campechanus*	+	18.88
R59	Tilapia	Online Retailer 11	Tilapia Fillet	*Oreochromis* spp.	*Oreochromis* spp.	*Oreochromis* spp.	-	-
R60	Flounder	Online Retailer 5	Wild caught Flounder - North Atlantic	Numerous possible species	*Hippoglossoides platessoides*	*Hippoglossoides platessoides*	-	-
R61	Pollock	Online Retailer 5	Wild caught Pollock - North Atlantic	*Pollachius virens*	*Pollachius virens*[a]	*Pollachius virens*[a]	-	-
R62	Red snapper	Online Retailer 5	Wild caught Red Snapper Fillets	*Lutjanus campechanus*	*Lutjanus peru*	*Lutjanus peru*	-	-
R63	Halibut	Online Retailer 5	Wild caught Halibut - North Atlantic	*Hippoglossus hippoglossus*	*Hippoglossus hippoglossus*[a]	*Hippoglossus hippoglossus*[a]	-	-
R64	Red snapper/other snappers	Online Retailer 12	Wild Caught Red Snapper Filets (online)/ *Pristipomoides* species (label)	*Lutjanus campechanus*	*Pristipomoides typus*[a]	*Pristipomoides typus*[a]	-	-
R65	Red snapper	Online Retailer 13	Fresh Wild Red Snapper Fillets	*Lutjanus campechanus*	*Ocyurus chrysurus*[a]	*Ocyurus chrysurus*[a]	-	-
R66	Red snapper	Online Retailer 14	Fresh Red Snapper	*Lutjanus campechanus*	*Rhomboplites aurorubens*[a]	*Rhomboplites aurorubens*[a]	-	-
R67	Red snapper	Online Retailer 15	Red Snapper	*Lutjanus campechanus*	*Lutjanus* spp.[ac]	*Lutjanus* spp.[ac]	-	-
R68	Red snapper	Online Retailer 16	Red Snapper Fillets	*Lutjanus campechanus*	*Lutjanus* spp.[ac]	*Lutjanus* spp.[ac]	-	-
R69	Perch	Online Retailer 17	Pacific Ocean Perch Fillets	*Sebastes alutus*	*Sebastes* spp.[a]	*Sebastes* spp.[a]	-	-
R70	Red snapper	Online Retailer 18	Red Snapper (Fillets)	*Lutjanus campechanus*	*Lutjanus campechanus, Lutjanus purpureus*	*Lutjanus campechanus*	+	16.48
R71	Tilefish	Online Retailer 18	Tilefish (Fillets)	*Lopholatilus chamaeleonticeps*	*Lopholatilus chamaeleonticeps*	*Lopholatilus chamaeleonticeps*	+/-	27.86/ -
R72	Snapper, vermilion	Online Retailer 18	Vermilion Snapper	*Rhomboplites aurorubens*	*Rhomboplites aurorubens*	*Rhomboplites aurorubens*	-	-

R73	Tilefish	Online Retailer 7	Fresh Ocean Whitefish Fillet (Tilefish)	*Caulolatilus princeps*	*Caulolatilus princeps*	*Caulolatilus princeps*	-	-
R74	Halibut	Online Retailer 7	Fresh Local Halibut Fillet	*Hippoglossus hippoglossus* or *Hippoglossus stenolepis*	No species match	*Paralichthys aestuarius*	-	-
R75	Red snapper	Online Retailer 6	Red Snapper Fillet Wild Fresh	*Lutjanus campechanus*	*Sebastes brevispinis*	*Sebastes brevispinis*	-	-
R76	Red snapper	Online Retailer 11	Red Snapper Fillets I.Q.F.	*Lutjanus campechanus*	*Lutjanus malabaricus*	*Lutjanus malabaricus*	-	-
R77	Red snapper	Online Retailer 3	Red Snapper Fillet	*Lutjanus campechanus*	*Lutjanus malabaricus*	*Lutjanus malabaricus*	-	-
R78	Red snapper	Online Retailer 9	Fresh Red Snapper	*Lutjanus campechanus*	*Coryphaena hippurus*	*Coryphaena hippurus*	+/+	19.06/ 20.41

[a]Sample was identified with DNA mini barcoding

[b]No information available from the FDA Seafood List

[c]Sample showed equivalent top matches to *Lutjanus malabaricus, Lutjanus erythropterus,* and *Lutjanus lutjanus* in both BOLD and GenBank, as well as to *Lutjanus gibbus* in BOLD

Table 3. Samples determined to be mislabeled (n = 31) due to species substitution and/or the use of an unacceptable market name according to the FDA Seafood List. In cases where a sample showed an equivalent top genetic match to multiple species and/or different species matches in BOLD and GenBank, all possible species are listed. Note: FDA recommends using the common name as the market name unless otherwise prohibited.

Sample	Category	Product name as advertised	Expected species	Identified species	Acceptable market name(s) of identified species (other than common name)	Notes
R53	Bass	Santa Barbara White Sea Bass	White seabass (*Atractoscian nobilis*)	Orangemouth weakfish (*Cynoscion xanthulus*)/ gray seatrout (*Cynoscion acoupa*)	Weakfish	Species substitution
R05	Cod	Fresh Wild Caught Pacific Cod Fillets	Pacific cod (*Gadus macrocephalus*)	Atlantic cod (*Gadus morhua*)	Cod	Species substitution
R11	Cod	True Cod Fish Fillet	Pacific cod (*Gadus macrocephalus*)	Pacific cod (*Gadus macrocephalus*)/Arctic cod (*Boreogadus saida*)/ Greenland cod (*Gadus ogac*)	Cod or Alaska cod	Unacceptable market name (true cod is a vernacular name for Pacific cod)
R48	Flounder	Flounder Fillet	Numerous possible species	Sutchi catfish (*Pangasianodon hypophthalmus*)/basa (*Pangasius bocourti*)/*Pangasius djambal*[ab]	Swai or sutchi or striped pangasius or tra or basa	Species substitution
R47	Flounder/ Halibut	Proton-Frozen Local Halibut Fillet (online)/ Frozen Flounder Fillet (label)	Atlantic halibut (*Hippoglossus hippoglossus*) or Pacific halibut (*Hippoglossus stenolepis*)/ Numerous possible flounder species	Speckled flounder (*Paralichthys woolmani*)[a]	N/A	Conflicting market names: Acceptable market name on product label but not on website.
R45	Halibut	Hirame Halibut Sashimi Live From Korea	Atlantic halibut (*Hippoglossus hippoglossus*) or Pacific halibut (*Hippoglossus stenolepis*)	Olive flounder (*Paralichthys olivaceus*)	Flounder	Species substitution (21 CFR 102.57)[c]
R55	Halibut	Local Halibut	Atlantic halibut (*Hippoglossus hippoglossus*) or Pacific halibut (*Hippoglossus stenolepis*)	California flounder (*Paralichthys californicus*)	Flounder	Species substitution (21 CFR 102.57)[c]

R74	Halibut	Fresh Local Halibut Fillet	Atlantic halibut (*Hippoglossus hippoglossus*) or Pacific halibut (*Hippoglossus stenolepis*)	Cortez halibut (*Paralichthys aestuarius*)	Flounder	Species substitution (21 CFR 102.57)[c]
R44	Perch	Akauo kirimi wild Alaska USA[b]	Numerous possible species	Shortspine thornyhead (*Sebastolobus alascanus*)/ longspine thornyhead (*Sebastolobus altivelis*)	Thornyhead	Unacceptable market name: Akauo is not an acceptable name for thornyhead.
R78	Red snapper	Fresh Red Snapper	Red snapper (*Lutjanus campechanus*)	Dolphinfish (*Coryphaena hippurus*)	Mahi-mahi	Species substitution
R41-42	Red snapper	Fresh Red Snapper Sashimi Farmed or Red Snapper from Japan	Red snapper (*Lutjanus campechanus*)	Madai (*Pagrus major*)/ squirefish (*Pagrus auratus*)	Porgy or sea bream	Species substitution
R43	Red snapper	Red Snapper Wild New Zealand	Red snapper (*Lutjanus campechanus*)	Madai (*Pagrus major*)/ squirefish (*Pagrus auratus*)	Porgy or sea bream	Species substitution
R46	Red snapper	Premium Red Snapper Japan Wild	Red snapper (*Lutjanus campechanus*)	Madai (*Pagrus major*)/ squirefish (*Pagrus auratus*)	Porgy or sea bream	Species substitution
R39	Red snapper	Red Snapper Fillet Wild Fresh	Red snapper (*Lutjanus campechanus*)	Yellowtail rockfish (*Sebastes flavidus*)	Rockfish	Species substitution
R75	Red snapper	Red Snapper Fillet Wild Fresh	Red snapper (*Lutjanus campechanus*)	Silvergray rockfish (*Sebastes brevispinis*)	Rockfish	Species substitution
R52	Red snapper/ Rockfish	Local Red Snapper/Rockfish	Red snapper (*Lutjanus campechanus*) or *Sebastes spp.*	Pacific Ocean perch (*Sebastes alutus*)	Rockfish or ocean perch	Unacceptable market name (combination of different species)
R49	Red snapper	Red Snapper Fillet	Red snapper (*Lutjanus campechanus*)	Crimson snapper (*Lutjanus erythropterus*)/ Malabar snapper (*Lutjanus malabaricus*)	Snapper	Species substitution
R20-21	Red snapper	Red Snapper (Sashimi grade)	Red snapper (*Lutjanus campechanus*)	Malabar snapper (*Lutjanus malabaricus*)	Snapper	Species substitution
R27, R76-77	Red snapper	Red Snapper Fillet or Red Snapper Fillets I.Q.F.	Red snapper (*Lutjanus campechanus*)	Malabar snapper (*Lutjanus malabaricus*)	Snapper	Species substitution

R62	Red snapper	Wild caught Red Snapper Fillets	Red snapper (*Lutjanus campechanus*)	Pacific snapper (*Lutjanus peru*)	Snapper	Species substitution
R67-68	Red snapper	Red Snapper or Red Snapper Fillets	Red snapper (*Lutjanus campechanus*)	Snapper species (*Lutjanus* spp.[d])	Snapper	Species substitution
R25, R66	Red snapper	Red Snapper or Fresh Red Snapper	Red snapper (*Lutjanus campechanus*)	Vermilion snapper (*Rhomboplites aurorubens*)	Snapper	Species substitution
R65	Red snapper	Fresh Wild Red Snapper Fillets	Red snapper (*Lutjanus campechanus*)	Yellowtail snapper (*Ocyurus chrysurus*)	Snapper	Species substitution
R64	Red snapper/other snappers	Wild Caught Red Snapper Filets (online)/ *Pristipomoides* species (label)	Red snapper (*Lutjanus campechanus*)/ Numerous jobfish or snapper species	Sharptooth jobfish (*Pristipomoides typus*)	Snapper or jobfish	Conflicting market names: Accurate name on product label but not on website.
R58	Red snapper/ yellowtail snapper	Yellowtail Red Snapper Fillet	Yellowtail snapper (*Ocyurus chrysurus*) or red snapper (*Lutjanus campechanus*)	Red snapper (*Lutjanus campechanus*)/Caribbean red snapper (*Lutjanus purpureus*)	Snapper	Unacceptable market name (combination of two species)

[a]There are no records in the FDA Seafood List for the following fish: akauo, *Cynoscion xanthulus, Pangasius djambal,* or *Paralichthys woolmani.*

[b]The term "catfish" is not legally allowed to be part of the market name of a fish unless it belongs to the Ictaluridae family (21 U.S.C. 343(t)).

[c]The term "halibut" can only be used to describe *Hippoglossus hippoglossus* or *Hippoglossus stenolepis* (21 CFR 102.57).

[d]Sample showed equivalent top genetic matches to *Lutjanus malabaricus, Lutjanus erythropterus,* and *Lutjanus lutjanus* in both BOLD and GenBank, as well as to *Lutjanus gibbus* in BOLD

Figure S1. Photograph of two fillets sold as "red snapper" (R51 and R78) that were received from an online retailer in the same packaging. R51 was authenticated as red snapper with DNA barcoding while R78 was identified as mahi-mahi.