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1	Species substitution and country of origin mislabeling of catfish products on the U.S.
2	commercial market
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22 Abstract

Catfish belong to the order Siluriformes and include both the Ictaluridae and Pangasiidae 23 families. However, U.S. labeling laws require only species of the family Ictaluridae to be 24 marketed as catfish. The lower production price of Pangasiidae, combined with changes in 25 regulations over time, have resulted in high potential for species substitution and country of 26 27 origin mislabeling among catfish products. The objective of this study was to conduct a market survey of catfish products sold at the U.S. retail level to examine species mislabeling and 28 compliance with Country of Origin Labeling (COOL) regulations. A total of 80 catfish samples 29 30 were collected from restaurants, grocery stores and fish markets in Orange County, CA. DNA was extracted from each sample and tested with real-time polymerase chain reaction (PCR) using 31 the InstantID[™] U.S. Catfish Assay Kit for Ictaluridae spp. (InstantLabs). Samples that tested 32 33 negative for Ictaluridae were tested with real-time PCR using the using the InstantID Asian Catfish Assay Kit for Pangasiidae spp. DNA barcoding was used as a final test in cases where 34 species could not be identified with either of the real-time PCR assays. Overall, 7 of the 80 of the 35 catfish products were found to be substituted with Pangasiidae species for a mislabeling rate of 36 9%. This included five of the 40 restaurant samples and two of the 32 grocery store samples. 37 Additionally, 59% of grocery store samples were not compliant with COOL regulations. The 38 results of this study reveal the occurrence of catfish mislabeling on the U.S. commercial market 39 40 and suggest the need for continuous monitoring of these products.

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42 Keywords: Catfish; DNA barcoding; Pangasius; real-time PCR; seafood fraud; country of origin

43 **1. Introduction**

Fisheries and aquaculture are an important source of food, nutrition, and income for 44 hundreds of millions of people globally. In 2014, the world *per capita* fish supply reached a new 45 high of 20 kg, attributed to the expanding growth in aquaculture which is responsible for half of 46 all fish for human consumption (FAO 2016). In the United States, over 90% of the seafood is 47 48 imported with over half of imports coming from aquaculture (NOAA 2016a). With this high percentage of foreign trade, an increase in seafood processing and consumer demand, and 49 globalization of the seafood industry, the potential for seafood fraud increases (Hellberg and 50 51 Morrissey 2011). Fish in their whole, unprocessed form are generally identifiable by morphological indicators. However, following processing it can be difficult to identify a species 52 by conventional taxonomic means. Seafood fraud, such as species substitution and mislabeling, 53 54 can occur at any stage along the supply chain from the initial production/capture to retail shops and restaurants. In the case of seafood substitution, a low-valued species is typically substituted 55 for a more expensive one while other types of seafood mislabeling, such as inaccurate country of 56 origin labeling, are committed to evade inspection, tariffs, and other costs (NOAA 2016a). 57 Accurate labeling of seafood is necessary to ensure food safety, avoid economic, social, and 58 59 conservation concerns, and truthfully inform consumers (Naaum et al. 2016). Country of Origin Labeling (COOL) is a labeling law that requires large retailers, such as 60

supermarkets, to provide information regarding method of production and country of origin
(Country of Origin Labeling for Fish and Shellfish, 7 C.F.R. § 60, 2009). COOL for covered fish
and shellfish commodities became effective in 2005 and is regulated by the USDA's Agricultural
Marketing Service (AMS). As part of COOL, fresh or frozen fish that have not undergone
transformation or processing outlined in 7 C.F.R. § 60 must be labeled with the name of the

66 country the fish is from and the method of production (wild-caught or farm-raised) (AMS 2017a). Wild-caught fish are those that are naturally-born or hatchery-originated that are released 67 in the wild and caught from non-controlled waters, while farm-raised fish are harvested in 68 69 controlled environments. Although food service establishments and fish markets may voluntarily include this information on the label, they are exempt from this ruling as they are not defined as 70 retailers under the Perishable Agriculture Commodities Act (1930; AMS 2017a). Similarly, 71 processed food items that have undergone specific processing resulting in a change in the 72 character of the commodity (e.g., cooked or smoked catfish) or those that have been combined 73 74 with at least one other covered commodity or food component (e.g., breaded catfish) are not subject to COOL. However, unless excepted by law, foreign articles imported into the United 75 States must be labeled with the correct country of origin according to 19 C.F.R. § 134.11 76 77 (Country of Origin Marking, 2011).

Catfish, order Siluriformes, represent more than 3,000 species, 477 genera, and 36 78 families (Ferraris 2007). In the U.S., the most commonly consumed species of Siluriformes are 79 from the Ictaluridae and Pangasiidae families (Delaware Sea Grant 2017). Ictaluridae catfish, 80 including blue catfish (Ictalurus furcatus) and channel catfish (Ictalurus punctatus), are the 81 82 leading aquaculture-produced seafood in the U.S., generating approximately half the freshwater aquaculture value in 2014 (NOAA 2016b). Ictaluridae catfish are also farm-raised in other 83 countries and imported into the United States, largely from China (NOAA 2018). Pangasius 84 85 catfish are part of the Pangasiidae family and include swai (Pangasianodon hypophthalmus; also known as tra or sutchi) and basa (*Pangasius bocourti*). These freshwater fish are primarily found 86 in the wild in South Asia and Southeast Asia and are farm-raised in a number of countries, 87 88 including Vietnam (Delaware Sea Grant 2017). Pangasius fish have been experiencing steady

demand globally, with the United States being the largest import market (FAO 2016). Vietnam
was the main source of imported Pangasius in the United States in 2016, with other sources
being Thailand and China (NOAA 2018). Pangasius fish are relatively low-priced (FAO 2016);
for example, one of the Southern California supermarket chains included in the current study
advertised prices of US\$4.99/lb (\$11.00/kg) for swai and US\$8.99/lb (\$19.82/kg) for U.S. catfish
in April 2018.

Vietnam began exporting Pangasius to the United States after the embargo on trade with 95 Vietnam was lifted in 1994 and exports grew tremendously following the removal of tariffs on 96 raw seafood in 1999 (Duc 2010). Swai and basa were initially marketed as "catfish" by 97 distributors in the U.S. However, with increasing competition from Vietnamese catfish imports, 98 the Association of Catfish Farmers of America (CFA) campaigned to require that Vietnamese 99 100 catfish be labeled as basa or swai to differentiate them from American catfish (Brambilla et al. 2012). In 2002, U.S. Congress passed a labeling law restricting the use of the name "catfish" 101 only to the Ictaluridae family (Duc 2010; Brambilla et al. 2012). These labeling restrictions were 102 103 incorporated into the United States Code under the Farm Security and Rural Investment Act (2002). However, passage of the labeling law did not lead to a significant recovery in U.S. 104 catfish prices, and CFA filed an antidumping lawsuit against Vietnam. In 2003, anti-dumping 105 duties were placed on imports of frozen swai and basa from Vietnam (DOC 2003). Since 2003, 106 several individuals and companies have been convicted of criminal charges related to falsely 107 108 mislabeling Vietnamese Pangasius as other species, such as grouper or sole, to avoid these tariffs (DOJ 2009; 2010; 2011). 109

Although most seafood is subject to periodic inspection by the U.S. Food and Drug
Administration (FDA), catfish are subject to continuous inspection by the United States

112 Department of Agriculture (USDA) Food Safety Inspection Services (FSIS) under the Federal Meat Inspection Act (FMIA), as required by the 2014 U.S. Farm Bill (FSIS 2015). The final 113 ruling released by FSIS regarding the catfish inspection program became effective in March 114 2016, with an 18-month transitional period until full enforcement in September 2017. According 115 to the 2014 Farm Bill, catfish subject to continuous inspection include all "fish of the order 116 117 Siluriformes." FSIS inspection procedures under the FMIA include verification that appropriate food safety standards and humane handling requirements are being followed. As part of the 118 catfish inspection program, lab samples may be periodically collected for analysis of chemical 119 120 residues, Salmonella, or speciation (FSIS 2018).

Existing literature on seafood fraud is extensive. Numerous studies have inspected the 121 mislabeling of various types of fish including salmon, tilapia, grouper, halibut, and pollock. 122 123 However, there is limited research specific to catfish mislabeling. In a market survey conducted by Consumer Reports, 3 of 21 "catfish" products purchased at retail outlets and restaurants in the 124 Northeastern United States were identified as swai with DNA testing (Consumer Reports 2011). 125 126 In a 2012 survey of seafood labeling at the wholesale distribution level, the FDA performed DNA barcoding on 40 fillets from 5 lots of domestic, channel catfish in California and reported 127 that none of the samples was mislabeled (FDA 2012). On the contrary, in a study conducted in 128 the Southeastern U.S., Wang and Hsieh (2016) reported that 26.7% of 15 "catfish" menu items 129 purchased from at restaurants were identified as Pangasius. According to the study authors, 130 131 Pangasius has the potential to be substituted for *Ictalurus* spp. because it is rapidly grown, produces a higher yield, and commands a lower price (Wang and Hsieh 2016). In a review of 132 seafood fraud reported globally, Pangasius was found to be one of the most commonly 133 134 substituted fish and was mislabeled as 18 different types of higher-valued species (Warner et al.

135 2016).

Due to the potential for catfish products to be mislabeled on the U.S. commercial market, the overall objective of this study was to investigate rates of species substitution and COOL compliance for catfish products sold at the retail level. Through a combination of real-time PCR and DNA barcoding, catfish products sold within the U.S. were analyzed to determine the occurrence of species substitution. Because the most common type of mislabeling expected was the substitution of Pangasius for *Ictalurus* spp., products were first tested for the presence of these species using real-time PCR, followed by DNA barcoding for any unidentified samples.

143 **2. Materials and Methods**

144 2.1 Sample collection and preparation

A total of 80 catfish products were purchased from locations in Orange County, 145 146 California, from July to August 2016. Forty of the products were purchased from 40 different restaurants and 40 products were purchased fresh/frozen from 39 different retail outlets (i.e., 8 147 fish markets and 31 grocery stores). All products purchased from grocery stores were subject to 148 149 COOL. Among the 31 grocery stores visited, 24 were supermarket chains and 7 were singlelocation supermarkets. Among fish markets, 1 was a chain and the other 7 were single-location 150 151 businesses. Out of the 40 restaurants visited, 13 were chains and 27 were single-location businesses. Only one location was visited for each chain store or restaurant chain included in this 152 study. Details about each sample were recorded, including cooking method, purchase location, 153 154 advertised name on the label or menu, production method, and country of origin labeling (if available). COOL compliance was assessed by examining the packaging labels for each product, 155 as well as all relevant labeling (e.g., placards, tags, signs, etc.) at the point of sale. Following 156 157 collection, samples were taken to the laboratory and prepared as described in Wang and Hsieh

(2016), with modifications. Batters, gravies, and sauces were removed from restaurant samples
using sterile deionized water. Similarly, fresh and frozen samples were rinsed with sterile
deionized water. After rinsing, approximately 5 g of tissue were removed from the interior of
each catfish sample using sterile forceps and scalpels. The 5 g sample was placed in a sterile 50
mL Falcon tube (Corning, Corning, NY) and stored at -80 °C until DNA extraction.

163 2.2 DNA extraction

DNA extraction was performed on tissue samples (~25 mg) using Qiagen's DNeasy
Blood and Tissue Kit, Spin Column Protocol (Qiagen, Valencia, CA), according to the
manufacturer's instructions. DNA was eluted in 50 µl Buffer AE preheated to 37 °C. The DNA
extract was used immediately for real-time PCR or stored at -20 °C for later use. A reagent blank
negative control with no sample tissue added was included alongside each set of extracted
samples. The DNA concentration was measured using a Thermo Scientific NanoDrop 2000
Spectrophotometer (Walham, MA).

171 2.3 *Real-time PCR*

A tiered approach was used to identify the species in each catfish sample. First, all 172 samples underwent real-time PCR with the InstantID[™] U.S. Catfish Assay Kit (InstantLabs, 173 174 Baltimore, MD). This kit tests for the presence of blue catfish (*Ictalurus furcatus*) or channel catfish (Ictalurus punctatus), with no differentiation between the two species. Any samples that 175 tested negative with the U.S. Catfish Assay were then tested with the InstantIDTM Asian Catfish 176 Assay (InstantLabs). This kit returns a positive result if basa (Pangasius bocourti) or swai 177 (*Pangasianodon hypophthalmus*) are present, with no differentiation between the two species. 178 Amplification was carried out using a Rotor-Gene® Q Cycler (Qiagen, Germantown, MD) and 179 each reaction tube included 12.5 µL 2X Master Mix (InstantLabs) and 12.5 µL DNA template 180

181 $(1.72 \pm 0.08 \,\mu\text{g})$. The 2X Master Mix provided with each kit included an internal control (IC). 182 Each kit also included positive control DNA (undiluted). Two, 10-fold serial dilutions of the positive control $(10^{-1} \text{ and } 10^{-2})$ were prepared using molecular-grade water. Each PCR run 183 included the undiluted positive control, the two positive control serial dilutions, and a negative 184 control with no DNA added. Thermocycler settings were followed according to InstantLabs: 95 185 °C for 5 min followed by 35 cycles of 95 °C for 10 s and 65 °C for 30 s. The results were 186 considered positive for a given sample if a cycle threshold (Ct) value was observed for the target 187 signal (FAM) and for the internal control signal (Cy5). The negative control was considered 188 189 valid if a Ct value was observed for the internal control but not for the target signal. 190 2.4 DNA-barcoding The single sample that tested negative with both the U.S. Catfish and the Asian Catfish 191 192 Assay Kits was next tested with DNA barcoding. PCR amplification of a 652-bp region of the cytochrome c oxidase subunit 1 (COI) gene was carried out using the C_FishF1t1-C_FishR1t1 193 primer combination described by Ivanova et al. (2007). This primer combination includes two 194 195 forward primers, VF2_t1 (5'-

TGTAAAACGACGGCCAGTCAACCAACCAACAAGACATTGGCAC-3') and FishF2_t1 (5' TGTAAAACGACGGCCAGTCGACTAATCATAAAGATATCGGCAC-3'), and two reverse
 primers, FishR2_t1 (5'-

199 CAGGAAACAGCTATGACACTTCAGGGTGACCGAAGAATCAGAA-3') and FR1d_t1 (5'-

- 200 CAGGAAACAGCTATGACACCTCAGGGTGTCCGAARAAYCARAA-3'). Each reaction
- tube included the following: 23 μL sterile H₂0, 25 μL HotStar Taq 2X Master Mix (Qiagen), 0.5
- μ L forward primers (10 μ M), 0.5 μ L reverse primers (10 μ M), and 1 μ L DNA template (0.12)
- 203 μg). Cycling conditions consisted of: 95 °C for 15 min, 35 cycles of 94 °C for 30 min, 52 °C for

40 s, and 72 °C for 1 min, with a final extension at 72 °C for 10 min. PCR was carried out with a
Mastercycler nexus gradient thermal cycler (Eppendorf, Hauppauge, NY) and a negative control
with no DNA added was included in the run.

207 PCR amplicon size and quality were confirmed with an E-Gel iBase Power System (Life Technologies, Carlsbad, CA). The PCR product $(4 \,\mu L)$ was loaded with 16 μL sterile water onto 208 a pre-cast 1% agarose E-gel (Life Technologies). The gel was run for 15 min and the results were 209 captured using Foto/Analyst Express (Fotodyne, Hartland, WI) combined with Transilluminator 210 FBDLT-88 (Fisher Scientific, Waltham, MA) and visualized with PCIMAGE (version 5.0.0.0 211 Fotodyne, Hartland, WI). The PCR product was stored at -20 °C until preparation for sequencing. 212 The PCR product was purified using the QIAquick PCR Purification Kit (Qiagen, Valencia, CA) 213 and the sample was shipped to GenScript (Piscataway, NJ) for bi-directional DNA sequencing 214 with the following M13 primers: M13F(-21) (5'-TGTAAAACGACGGCCAGT-3') and M13R(-215 27) (5'-CAGGAAACAGCTATGAC-3'). 216 2.5 Sequencing analysis 217

Raw sequence data was assembled and trimmed to the COI degenerate bony fish
barcoding sequence FISHREF08a (Handy et al. 2011) using Geneious R7 (Biomatters Ltd.,
Auckland, New Zealand). This sequence was identified to the species level using the Barcode of
Life Database (BOLD), Species Level Barcodes Records option, with a species-level cut off of ≥
98% genetic similarity. The common name for the identified species was determined using the
FDA's Guide to Acceptable Market Names for Seafood sold in Interstate Commerce (FDA
2016).

225 2.6 Follow-up testing

Establishments that were found to have products mislabeled based on species were revisited approximately one year following the initial collection. If the same product type was available, it was purchased and re-tested for species mislabeling using the tiered approach described above.

230 **3. Results and Discussion**

231 3.1 DNA-based test results

Out of the 80 samples collected, 73 were found to contain Ictaluridae species (Table 1). 232 Initially, 72 of the samples tested positive for Ictaluridae species with real-time PCR. Seven of 233 234 the eight samples that tested negative for Ictaluridae were found to be positive for Pangasiidae species through real-time PCR. The target signal Ct values for the positive controls used in the 235 U.S. Catfish and Asian Catfish real-time PCR assays ranged from 24.07 (undiluted) to 34.69 236 237 (1:100 dilution) whereas the target signal Ct values for samples ranged from 18.25 to 32.48. The average U.S. Catfish Ct values across the different sample types ranged from 20.83 for the one 238 steamed sample that tested positive with this kit to 22.74 ± 1.74 for pan-fried samples. The 239 240 sample that tested negative with both assays was a dish of grilled catfish purchased at a restaurant. DNA barcoding analysis of this sample resulted in a single forward sequence read 241 242 that was 535 bp in length and had 14.4% high quality bases. This sequence was identified as channel catfish with a genetic similarity of 99.1%. However, the DNA sequence did not meet the 243 quality parameters established by Handy et al. (2011) for DNA barcoding of fish for regulatory 244 purposes, which state that single sequence reads must have $\geq 98\%$ high quality bases. After 245 repeating DNA extraction and real-time PCR on this sample, it tested positive for Ictaluridae, in 246 247 agreement with the sequencing results.

248 3.2 Species mislabeling

249 Overall, 7 of the 80 products (9%) tested in the current study were determined to be 250 mislabeled with regard to species (Table 1). All seven mislabeled products were purchased from different locations and were found to contain Pangasiidae species in place of Ictaluridae species. 251 As noted in the Introduction, products labeled as catfish that are sold in the United States can 252 only contain species from the Ictaluridae family. Among the mislabeled restaurant dishes, one 253 was purchased from a local restaurant chain and four were purchased from single-location 254 businesses. The two mislabeled fresh/frozen products were purchased from seafood counters at 255 two different ethnic chain stores. Interestingly, the rate of species mislabeling among restaurant 256 257 dishes (12.5%) was higher than that found for fresh/frozen fish samples (5%). This is in agreement with the notion that fish with a higher degree of processing are more susceptible to 258 food fraud (Stiles et al. 2011). Along these lines, deep-fried fish were the most common 259 260 restaurant dish found to be mislabeled, with 4 of 22 deep-fried samples found to contain Pangasiidae instead of Ictaluridae. Two of the fraudulent dishes were labeled as "fried catfish 261 basket," one was labeled as "spicy catfish," and another was labeled as "fried catfish." Species 262 263 mislabeling was also detected in one steamed product labeled as "garlic catfish." Interestingly, deep-fried and steamed catfish were, on average, the least expensive restaurant dishes. These 264 dishes had average prices of ~US\$13 each, ranging from US\$7.49 to US\$20.47 for deep-fried 265 dishes and US\$12.00-US\$13.99 for steamed dishes. None of the pan-fried, grilled, or baked 266 products was found to be mislabeled on the basis of species. The baked samples were the most 267 268 highly valued, with an average price of US (range: US (22.00-49.14). However, all three baked catfish dishes purchased were sold as whole fish (head and skin on), thereby 269 reducing the potential for species mislabeling. 270

271 In the case of fresh/frozen samples, all nuggets, cuts and whole catfish were found to contain accurate species labeling. The whole catfish products had the head and skin on, thereby 272 exposing morphological indicators including color and barbels and making it more difficult to 273 274 deceive buyers. On the other hand, 2 of the 18 catfish fillets were found to contain Pangasiidae species. Fillets had the highest average price for fresh/frozen samples, at US 3.63 ± 1.27 per 8-275 276 oz (266.8-g) serving, compared to <US\$2.00 per 8-oz serving for whole catfish, nuggets, and cuts, indicating species substitution is more common in higher-valued fresh/frozen catfish 277 products. Both mislabeled fillets were purchased from seafood counters at grocery stores. One of 278 279 the fillets was labeled as "catfish" and the other was labeled "Filette de Pescado" but was verbally declared to be catfish by an employee. The only other sample collected in this study that 280 relied on a verbal declaration only was a sample of grilled catfish that was verified as containing 281 282 Ictaluridae.

Follow-up sampling and testing on the mislabeled catfish products was conducted 283 approximately one year after the initial collection date. The two products sold at grocery stores 284 were no longer available and one of the restaurants that sold mislabeled catfish was permanently 285 closed. The four remaining restaurant samples, consisting of four deep-fried products, were 286 287 available for recollection and retesting. All four samples were again found to be mislabeled, testing positive for Pangasiidae. These results indicate a recurring problem of species 288 mislabeling at these establishments; however, additional research is required to determine 289 290 whether the mislabeling is occurring at the restaurant level or earlier in the supply chain.

The species mislabeling rate of 12.5% for restaurant dishes in the current study is lower than that found by the study conducted by Wang and Hsieh (2016), which reported a mislabeling rate of 27% for restaurant dishes labeled as catfish in the Southeastern U.S. The study reported

294 that 4 of 15 catfish dishes tested were identified as Pangasius using enzyme-linked immunosorbent assay (ELISA). In comparison, the market survey conducted by Consumer 295 Reports (2011) in the Northeastern United States reported a catfish mislabeling rate of 14.3% 296 among a set of 21 products purchased at retail stores and restaurants. Aside from the differences 297 in sample size and geographic location, a possible explanation for the higher mislabeling rates 298 observed in these studies is that they were conducted prior to the release of the final ruling 299 establishing a continuous USDA inspection program for Siluriformes, including catfish (FSIS 300 2015). Prior to the ruling, catfish were under the jurisdiction of the FDA and were not subject to 301 302 continuous inspection. In comparison, the current study was conducted during the 18-month transitional period between the effective date of the final ruling (March 2016) and full 303 enforcement (September 2017). 304

In contrast to the above studies, a 2012 FDA survey did not find any mislabeling of catfish collected at the wholesale distribution level in California (FDA 2012). The FDA survey analyzed 40 fillets chosen at random from 5 lots of domestic catfish using DNA barcoding. The reduced mislabeling rate found by FDA may explained by differences in the study design, such as sample number and testing at the wholesale vs. retail level.

310 3.3 *COOL compliance*

In addition to species mislabeling, all fresh/frozen catfish products from grocery stores (n = 32) were surveyed for compliance with COOL (Table 2). To convey COOL information to consumers, information on the country of origin and production method for each product must be legible and placed in a location that can be read and understood, for example on a placard, sign, sticker, band, or twist tie (AMS 2017a). A total of 19 of the 32 fresh/frozen products (59%) were missing country of origin information, production method, or both from the label, meaning they 317 were not compliant with COOL. Among the products purchased from chain store locations, 52% (13 of 25 products) were not compliant with COOL, while 86% (6 of 7) products purchased at 318 single-location stores were not COOL compliant. Overall, 9 samples were missing country of 319 320 origin labeling and 1 sample contained information that was not compliant with COOL. This sample was a whole catfish labeled "Product of Ecuador/Thai/ or China" with no information on 321 322 the production method. This product tested positive for Ictaluridae species with real-time PCR. While Ictaluridae species are legally imported into the U.S. from other countries, labeling 323 country of origin with "or", "and/or", or "may contain" is not acceptable under COOL regulation 324 325 as specific origin information is not transparent to consumers (Country of Origin Labeling for Fish and Shellfish 2009). The 22 samples that contained country of origin information in 326 compliance with COOL were all labeled as products of the U.S. and tested positive for 327 328 Ictaluridae species.

A greater proportion of fresh/frozen grocery store samples (50%) was missing 329 information on the production method as compared to those that were non-compliant with 330 331 country of origin information (31%) (Table 2). All 16 samples that did include production method listed "farm-raised" on the label. As shown in Fig. 1, catfish nuggets had the highest rate 332 (57%, 4 of 7 samples) of labeling both country of origin and method of production, making these 333 the most COOL-compliant catfish product. Catfish fillets had the second highest rate (50%, 7 of 334 14 samples) of COOL compliance, and had the most diversity in terms of labeling, with samples 335 336 ranging from listing no COOL information to country of origin only, production method only, or both. Whole catfish products were found to be the least compliant with COOL, as only 1 of 8 337 samples (12.5%) contained both country of origin and method of production on the label. Fillets, 338 whole catfish, and cuts that were not COOL compliant were more likely to label country of 339

origin than production method, while nuggets were more likely to label production method.
Interestingly, both fillets determined to be mislabeled on the basis of species were also not
compliant with COOL. While no production method was given for either, one fillet was labeled
as "Product of the U.S." and the other fillet did not contain the country of origin information.
Pangasius is not produced in the United States, meaning that the country of origin information
was incorrect for the one fillet that listed it.

The percentage of fresh/frozen grocery store samples found to be non-compliant with 346 COOL in this study (59%) was higher than the overall rate reported by the COOL Division as a 347 348 result of their 2016 retail surveillance reviews (AMS 2017b). These reviews revealed that 10% of 17,928 fish and shellfish items sold from 3,087 retail stores in all 50 states were not compliant 349 with COOL (K. Becker, personal communication, June 21, 2017). However, information is not 350 351 available on individual species, making it difficult to make a direct comparison for catfish mislabeling. Further research is necessary to discern whether the lack of COOL compliance 352 observed in this study is restricted to catfish or is also observed in other fish species sold in this 353 354 sampling region. Similar to the results of this study, the AMS data revealed that a greater proportion of noncompliant samples were missing production information (55%) as compared to 355 356 country of origin (45%). The percentage of fresh/frozen grocery store samples found to be noncompliant with COOL in this study (59%) was also high compared to a previous COOL study 357 conducted in Baltimore, MD (Lagasse et al. 2014). Lagasse et al. (2014) reported that only 3.8% 358 of the 628 fresh/frozen seafood products examined in their study were missing production 359 method and/or country of origin information and an additional 1.9% of products listed multiple 360 origins. However, these numbers were based on data gathered at eight different retail outlets that 361 362 were visited approximately four times each. In comparison, the COOL results reported in the

current study were based on single visits to 31 different retail outlets. Interestingly, all of the 67
catfish samples analyzed by Lagasse et al. 2014 contained both production method and country
of origin information, with three of the samples listing multiple origins.

Although fish markets and restaurants are exempt from COOL, they can participate on a 366 voluntary basis. Table 2 shows a summary of COOL compliance for catfish products purchased 367 368 at these establishments. Among the eight products purchased from fish markets, only two fillets were COOL compliant, listing both country of origin (Product of the U.S.) and production 369 method (farm-raised). Similarly, of the 40 restaurant samples collected, two contained 370 371 information regarding country of origin (Product of the U.S.) and one included information 372 regarding production method (farm-raised). Additionally, one restaurant sample listed both country of origin (Product of the U.S.) and production method (farm-raised) making it COOL 373 374 compliant. The six products from fish markets and restaurants that supplied information regarding country of origin were all correctly identified as Ictaluridae species. While no fish 375 market samples were mislabeled in terms of species, the rate of species mislabeling among 376 377 restaurant samples that did not supply COOL information was 13%.

378 **4.** Conclusion

This study revealed mislabeling of catfish products sold in restaurants, grocery stores, and fish markets in Orange County, CA. Despite government regulations to prevent misbranding of food products, it is apparent that some catfish products are mislabeled through species substitution and/or by not labeling country of origin and method of production. Accurate labeling of seafood products is important not only for food safety, economic, and conservation reasons, but also to help consumers make informed buying decisions. The high rate of COOL noncompliance as well as evidence of catfish species substitution observed signify the importance of

continuous monitoring of catfish products for mislabeling. The rapid real-time PCR assay
utilized in this study could serve as a useful tool for routine monitoring by regulatory bodies and
the seafood industry when testing species authenticity of catfish. Additional market research on
catfish mislabeling within the United States is recommended in order to determine steps to
reduce species substitution and to improve COOL compliance.

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Product type		Number of products collected	Number of products identified as Ictaluridae	Number of products identified as Pangasiidae	Average cost \pm SD (USD) ^a	Price range (USD) ^a
Restaurant	Deep fried	22	18	4	13.45 ± 3.75	7.49-20.47
dishes	Pan-fried	7	7	0	16.83 ± 5.77	10.28-25.75
	Grilled	6	6	0	13.51 ± 2.33	9.67-14.60
	Baked	3	3	0	34.38 ± 13.73	22.00-49.14
	Steamed	2	1	1	13.00 ± 1.41	12.00-13.99
	Overall	40	35	5	15.60 ± 7.36	7.49-49.14
Fresh/frozen	Fillets	18	16	2	3.63 ± 1.27	1.75-5.48
fish	Whole fish, head on	11	11	0	1.69 ± 0.24	1.50-2.00
	Nuggets ^b	8	8	0	1.52 ± 0.07	1.50-1.65
	Cuts ^c	3	3	0	1.62 ± 0.53	1.25-2.00
	Overall	40	38	2	2.47 ± 1.32	1.50-5.48
All products combined 80		80	73	7	11.08 ± 8.68	1.50-49.14

Table 1. Summary of catfish products collected for this study and results of DNA testing.

^a Missing price data for nine fillets, seven whole catfish, two nuggets, and one cut. Fresh/frozen prices are expressed as per 8-oz (226.8-g) serving of fish.

^bNuggets are defined as pieces of belly flaps with or without black membrane and weighing not less than ³/₄ ounce or 21.3 g (NOAA 2017).

^cCuts are defined as fillet cuts or steaks with or without bone (NOAA 2017).

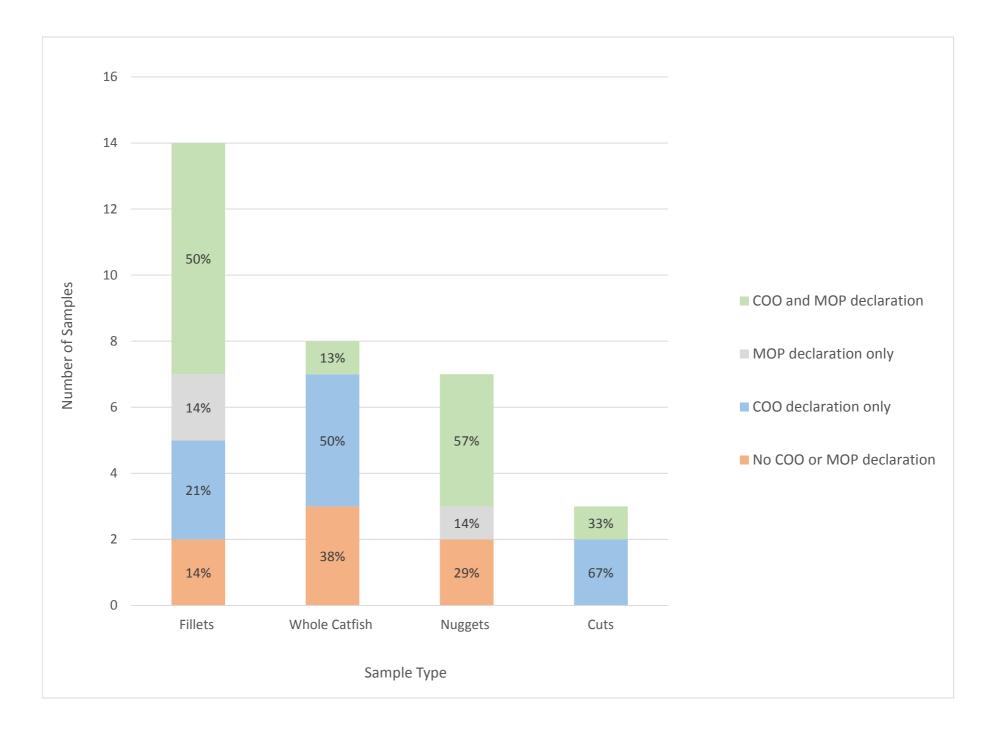
Purchase location*	Number of samples	COOL noncompliant	No or incorrect MOP declaration	No or incorrect COO declaration	Neither COO or MOP declared
Restaurant	40 (50%)	39 (97.5%)	38 (95%)	37 (92.5%)	36 (90%)
Grocery Store	32 (40%)	19 (59.4%)	16 (50%)	10 (31.3%)	7 (21.9%)
Fish Markets	8 (10%)	6 (75%)	6 (75%)	6 (75%)	6 (75%)
Total	80	64 (80%)	61 (76.3%)	55 (68.8%)	49 (61.3%)

Table 2. Summary of COOL noncompliance for catfish products tested in this study, including information on method of production (MOP) and country of origin (COO) declarations. Values are displayed as the number count (percentage of total).

*Compliance with COOL is voluntary for restaurants and fish markets

Figure Captions

Figure 1. Summary of COOL compliance for fresh/frozen fish samples (n = 32) collected from grocery stores, including information on method of production (MOP) and country of origin (COO) declarations.



	Fillets	Whole Catfish	Nuggets
No COO or MOP declaration	2	3	2
COO declaration only	3	4	0
MOP declaration only	2	0	1
COO and MOP declaration	7	1	4
Total	14	8	7
	Fillets	Whole Catfish	Nuggets
No Country of Origin or Production Method	14%	38%	29%
Country of Origin Only	21%	50%	0%
Production Method Only	14%	0%	14%
Country of Origin and Production Method	50%	13%	57%
Total	100%	100%	100%

Fresh/Frozen Samples (Grocery Store) FIG 1

0 2 0
1
3
5
Cuts
0%
67%
0%
33%
100%

Cuts