



Using DNA barcoding to track seafood mislabeling in Los Angeles restaurants

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Abstract: Seafood mislabeling is common in both domestic and international markets. Studies on seafood fraud often report high rates of mislabeling (e.g., >70%), but these studies have been limited to a single sampling year, which means it is difficult to assess the impact of stricter governmental truth-in-labeling regulations. We used DNA barcoding to assess seafood labeling in 26 sushi restaurants in Los Angeles over 4 years. Seafood from 3 high-end grocery stores were also sampled (n = 16) in 2014. We ordered 9 common sushi fish from menus, preserved tissue samples in 95% ethanol, extracted the genomic DNA, amplified and sequenced a portion of the mtDNA COI gene, and identified the resulting sequence to known fish sequences from the National Center for Biotechnology Information nucleotide database. We compared DNA results with the U.S. Food and Drug Administration (FDA) list of acceptable market names and retail names. We considered sushi-sample labels that were inconsistent with FDA names mislabeled. Sushi restaurants had a consistently high percentage of mislabeling (47%; 151 of 323) from 2012 to 2015, yet mislabeling was not homogenous across species. Halibut, red snapper, yellowfin tuna, and yellowtail had consistently high (<77%) occurrences of mislabeling on menus, whereas mislabeling of salmon and mackerel were typically low (>15%). All sampled sushi restaurants had at least one case of mislabeling. Mislabeling of sushi-grade fish from high-end grocery stores was also identified in red snapper, yellowfin tuna, and yellowtail, but at a slightly lower frequency (42%) than sushi restaurants. Despite increased regulatory measures and media attention, we found seafood mislabeling continues to be prevalent.

Keywords: cytochrome oxidase subunit I (COI), halibut, seafood fraud, species substitution, sushi, tuna
Utilización del Código de Barras de ADN para Rastrear Pescados Mal Etiquetados en Restaurantes de Los Ángeles

Resumen: La mala etiquetación de pescados es común tanto en los mercados domésticos como en los internacionales. Los estudios sobre el fraude de pescados generalmente reportan tasas altas de mala etiquetación (p. ej.: > 70 %), pero estos estudios han sido limitados a un sólo muestreo al año, lo que significa que es complicado evaluar el impacto de regulaciones gubernamentales más estrictas sobre las etiquetas verdicas. Utilizamos el código de barras de ADN para evaluar el etiquetado de pescados en 26 restaurantes de sushi en Los Ángeles durante cuatro años. Los pescados de tres supermercados lujosos también fueron muestreados (n = 16) en el 2014. Ordenamos nueve pescados comunes en el sushi de los menús, preservamos las muestras de tejido en etanol al 95 %, extrajimos el ADN genómico, amplificamos y secuenciamos la porción del gen COI del ADNmt, e identificamos la secuencia resultante a partir de secuencias de peces de la base de datos de nucleótidos del Centro Nacional para la Información Biotecnológica. Comparamos los resultados de ADN con la lista de nombres aceptables para el mercado y de venta al menudeo de la Administración Estadunidense de Alimentos y Medicamentos (FDA, en inglés). Consideramos como mal etiquetadas a las muestras de sushi que no fueron consistentes con los nombres de la FDA. Los restaurantes de sushi tuvieron constantemente un porcentaje alto de mala etiquetación (47 %; 151 de 323) de 2012 a 2015, sin embargo, la mala etiquetación

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no fue homogénea entre las especies. El hipogloso, el buachinango, el atún de aleta amarilla y el jurel tuvieron ocurrencias altas (<77 %) de mala etiquetación en los menús, mientras que la mala etiquetación del salmón y la caballa fue típicamente baja (>15 %). Todos los restaurantes de sushi muestreados tuvieron por lo menos un caso de mala etiquetación. La mala etiquetación de pescado con calidad para sushi de los supermercados lujosos también fue identificada para el buachinango, el atún de aleta amarilla y el jurel, pero a una frecuencia un poco menor (42 %) que en los restaurantes de sushi. A pesar del incremento en las medidas regulatorias y en la atención de los medios, encontramos que la mala etiquetación de los pescados todavía es prevalente.

Palabras Clave: atún, fraude de pescados, hipogloso, subunidad citocromo oxidasa (COI), sushi, sustitución de especies

Introduction

Seafood fraud is prevalent in both the international and domestic U.S. fish trade. Fraud occurs when seafood is sold under something other than its true species name (U.S. Food and Drug Administration [FDA] 2015). Incorrect attribution of species names in seafood products is referred to as species substitution, which often goes unnoticed because it is difficult to authenticate the identity of species once they are in the supply chain.

Mislabeled can be unintentional, resulting from species misidentification, incorrect assignment of a common vernacular name (Buck 2010), or the loss of product information during exchanges within the supply chain (Cohen et al. 2009). Deliberate mislabeling is used to increase profit and introduce illegally captured fish into legal trade (Ogden 2008; Cawthorn et al. 2012). Mislabeled can happen at any point in the supply chain, from fisher to retailer; thus, determining how substitutions occur is complicated.

Opportunity for seafood fraud is increasing. Global per capita consumption of seafood is over 20 kg (FAO 2016), and approximately 4.5 billion people depend on seafood for nutrition and livelihood (Béné et al. 2015). Nearly 40% of the world's captured seafood is traded internationally (Tveteras et al. 2012); global fish trade is valued at over \$135 billion (FAO 2016). The industry's development and complexity has outpaced the ability to monitor the trade. Improved traceability and accuracy of seafood labeling through amendments to existing enforcement measures (e.g., Food and Agriculture Organization Amendment on Port State Measures on IUU, 2015) and regulations incorporated into new trade agreements (e.g., TPP environmental provisions, 2016) are being developed and implemented. However, determining the impact of such actions is challenging because once a fish enters the supply chain labeling is often poorly tracked.

Harm of Seafood Fraud

Seafood fraud weakens public trust of the seafood trade and adds pressure to already overharvested fisheries (Pauly et al. 2005). For example, red snapper (*Lutjanus campechanus*) remains among the most valued fisheries

in the Gulf of Mexico, despite having been pronounced overfished in the mid-1990s (Marko et al. 2004). Increasingly stringent regulations have incentivized species substitution for red snapper, resulting in rampant mislabeling (over 70%). Such mislabeling can substantially delay fisheries management action (Garcia & Charles 2008) and distort consumer perceptions that fish supply meets market demands (Marko et al. 2004). In contrast, scientific investigations of seafood fraud paired with public outreach reduced mislabeling frequency of Atlantic cod (*Gadus morhua*) in Europe and increased pressure on fraudulent seafood practices (Mariani et al. 2014; Naaum & Hanner 2015).

Seafood mislabeling also undermines the effectiveness of certification programs aimed at increasing consumer awareness and decreasing demand for unsustainable species. Certification programs have risen dramatically in recent years as consumer demand for natural, organic, and ecofriendly products have risen worldwide (Schleenbecker & Hamm 2013; Rousseau 2015), particularly within the fisheries sector (Gulbrandsen 2009; Uchida et al. 2014). Starting in the 1990s, increasing public awareness and concern over dolphin bycatch in tuna fisheries pushed forward legislation for dolphin-safe labels and harvest practices (e.g., Dolphin Protection Consumer Information Act 16 US Code 1385). More recently, sustainable seafood guides and certifications such as the Monterey Bay Aquarium's Seafood Watch program, Seafood Choice Alliance, and the Marine Stewardship Council have become important consumer resources. Furthermore, the FDA Seafood List provides acceptable and common market names for seafood sold in interstate commerce and information on pertinent regulations and food-safety hazards. The success of these programs relies on accurate labeling and traceability of seafood products throughout the supply chain so that consumers can make informed decisions.

Seafood fraud is also a critical health concern that compromises consumers' ability to adhere to dietary restrictions, make ethical personal food choices, and, in severe instances, may threaten human life. For example, mislabeling of oilfish (*Ruvettus pretiosus*) and escolar (*Lepidocybium flavobrunneum*) has circumvented bans and restrictions on these fishes in many countries, leading

Table 1. Rates of seafood mislabeling reported in recent DNA barcoding studies in which the genetic marker was cytochrome oxidase subunit I.

Country	Sampling year (sampling periods) ^a	n	Mislabeling (%)	Sample source ^b	Taxon surveyed	Reference
Canada and United States	NR	91	25	R, FM	diverse	Wong & Hanner 2008
Canada	2012-2013 (1)	293	23	NR	diverse	Naaum & Hanner 2015
England	2008-2009 (1)	212	<1.5	S	fish sticks	Huxley-Jones et al. 2012
England	NR	371	6	S	cod, haddock, pollock	Helyar et al. 2014
Europe	2013-2014 (1)	1,563	5	S, FM	diverse	Mariani et al. 2015
France	2013 (1)	371	4	R, S, FM	diverse	Bénard-Capelle et al. 2015
Ireland	2009 (1)	156	25	R, S	cod, haddock	Miller & Mariani 2010
Ireland	2011 (1)	66	15	R, S	cod	Mariani et al. 2014
Italy	NR	45	77	FM	sharks	Barbuto et al. 2010
Philippines	2010-2011 (1)	14	79	S, FM	sardine, tuna, dory	Maralit et al. 2013
South Africa	2008-2010 (1)	248	21	S, FM	diverse	Cawthorn et al. 2012
United States	2011 (1)	119	55	R, S	diverse	Warner et al. 2012
United States	2014 (1)	172	16	R, S	diverse	Khaksar et al. 2015
United States	2003 (1)	22	77	FM	red snapper	Marko et al. 2004
United States	2012-2015 (4)	323	47	R	diverse	this study
United States	2014 (1)	14	42	S	diverse	this study

^aAbbreviation: NR, data not reported or specifically stated in the study.

^bAbbreviations: R, restaurant; FM, fish market or fishmonger; S, supermarket.

to widespread and frequent outbreaks of keriorrhea after their consumption (Ling et al. 2008). The mislabeling of the pufferfish (*Lagocephalus* sp.) as monkfish resulted in the hospitalization and temporary neurological damage of consumers and the recall of monkfish in three U.S. states in 2007 (Cohen et al. 2009). Finally, substitution among tuna species in canned tuna resulted in increased mercury levels in canned so-called light tuna, prescribed as a relatively safe dietary alternative to other canned tuna for children and pregnant women (Burger & Gochfeld 2004; Jacquet & Pauly 2008).

DNA Barcoding and Mislabeling Rates Over Time

Over the past decade, DNA barcoding has become an increasingly popular tool to identify mislabeled products. In DNA barcoding a partial DNA sequence of the mitochondrial COI gene is used as a diagnostic marker to identify tissue samples to species (Hebert et al. 2003). Use of DNA barcoding to investigate seafood fraud is widespread and has yielded a range of mislabeling frequencies (Table 1) that vary by retailer type. Rates are lowest among grocers and highest in restaurants (e.g., Warner et al. 2012; Bénard-Capelle et al. 2015; Khaksar et al. 2015).

Although most researchers report mislabeling data from a single sampling period, a few have examined seafood mislabeling rates over time (Table 1). Generally, these studies compared data from geographically different regions and collected with a range of sampling methods. An exception is Mariani et al. (2014), who compared cod-mislabeling frequencies from 2009 to 2011 and found mislabeling decreased in supermarkets over

time (35–0%) but changed minimally in takeaway vendors (50–42%). Such longitudinal studies are essential to evaluating changes over time in consumer awareness and to testing the effectiveness of labeling regulations and providing better overall estimates of mislabeling.

We evaluated the extent of seafood mislabeling over 4 years in the metropolitan area of Los Angeles, California (U.S.A.). We used DNA barcoding to target nine popular fish species from multiple sushi restaurants, a retailer type shown to have a high incidence of mislabeling (58% in Warner et al. [2012]). We assessed species' substitution rates over time to provide a multiyear perspective of the state of seafood mislabeling ahead of impending enforcement of new U.S. seafood-labeling regulations.

Methods

Sample Collection

Students in Introduction to Marine Science at the University of California, Los Angeles (UCLA), collected 364 fish samples from 26 sushi restaurants in Los Angeles, California, that received high or good customer scores on 2 popular online restaurant rating services. We sampled 69% of the restaurants in 2 or more years. We targeted 9 popular sushi fish: albacore tuna (*Thunnus alalunga*), yellowfin tuna (*T. albacares*), bigeye tuna (*T. obesus*), bluefin tuna (*T. thynnus*, *T. maccoyii*, *T. orientalis*), red snapper, yellowtail (*Seriola lalandi*), halibut (*Hippoglossus hippoglossus*, *H. stenolepis*), Mackerel (*Scomber* spp., *Scomberomorus* spp.), and

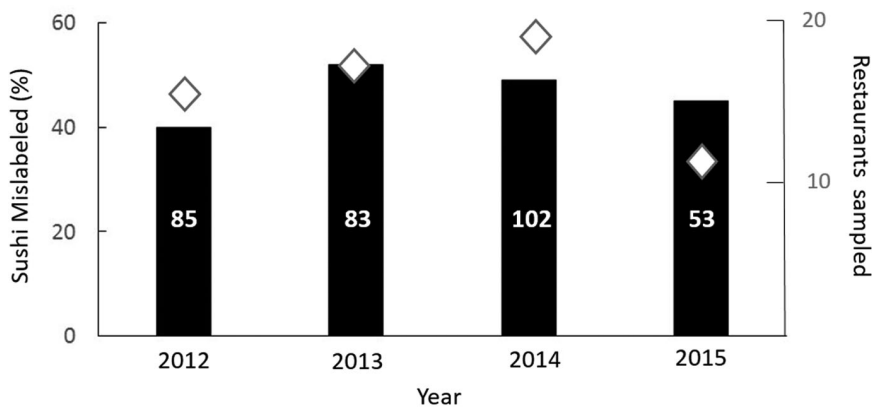


Figure 1. Frequency of mislabeling of sushi fish type by year (numbers in bars, total number of samples per year; diamonds, number of sushi restaurants sampled).

salmon (*Salmo salar*, *Oncorhynchus* spp.). Given the ambiguity in menu names, a tenth group recorded generally as tuna (*Thunnus* spp.) was included in the sampling. Students ordered these fish from sushi menus, confirmed the identity of the sushi with the wait staff, and then collected a small tissue sample with sterile instruments, preserving the sample in 95% ethanol for future analysis. Each fish type was sampled only once per restaurant per year. To compare sushi restaurants with fish retailers, in 2014 we sampled sushi-grade filets ($n = 16$) of the target fish from 3 upscale grocery stores.

DNA Barcoding

We extracted genomic DNA from approximately 25 mg of tissue with a 10% Chelex solution (BioRad, Irvine, CA, USA) (Walsh et al. 1991). We then amplified an approximately 650-bp fragment of the mitochondrial COI gene with a primer cocktail, C.FishF1t1 and C.FishR1t1 (Ivanova et al. 2007). The reaction mixture, 25 μ L total volume, included 1 μ L of gDNA, 1.25 μ L of 10MM of each primer, and 19 μ L of molecular grade water in Illustra PuRe Taq Ready-To-Go polymerase chain reaction (PCR) bead 0.2 mL tubes (GE Lifesciences, Pittsburgh, PA, USA). Thermal cycling began with an initial denaturing at 94°C for 5 min that was followed by 35 cycles of denaturing at 94°C for 30 s, annealing at 50°C for 45 s, and extension at 72°C for 60 s, with a final extension at 72°C for 10 min. We visualized PCR products on a 1% agarose gel via gel electrophoresis and sent successful double-stranded amplicons for purification and sequencing with the M13 primer pair (Messing 1983) at the University of California Berkeley DNA Sequencing facility.

Data Analyses

We proofread and assembled double-stranded sequences with Geneious 8.1.7 (BioMatters, Auckland, New Zealand) software (Kearse et al. 2012) and identified resulting consensus sequences to the lowest taxonomic level with the Basic Local Alignment Search Tool (BLAST) on the Nation Center for Biotechnology Information

(NCBI) website (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). We used the MegaBLAST option to optimize the database search for highly similar sequences. A 98% cut-off for nucleotide homology was used to determine a match. In cases of multiple strong matches, we used the maximum score for final sample identification. We then compared the DNA barcode identification with the labeled menu or retail name (point-of-sale name) for each sample, which we in turn compared with the FDA Seafood List of acceptable market common names for each identified species. We recorded sushi samples with labeled names that were inconsistent with FDA accepted market names as mislabeled. For the general tuna group, we counted any match to a known *Thunnus* sequence as accurate. We used pairwise comparisons of mislabeling percentages for combined taxa to test for significant differences among sampling years. To further test for an association between individual fish sample groups and each year, we used a Fisher exact probability test and then assessed the effect of sampling effort with Spearman's rank correlation coefficient.

Results

Seafood Identification

We obtained successful PCR and sequence data from 323 of 364 samples (89%). Forty-seven percent (151 of 323) of samples were mislabeled. Yearly mislabeling frequencies ranged from 40% to 52% (Fig. 1). Pairwise comparison between mislabeling percentages was not significantly different among sampling years ($\chi^2 = 2.67$, $df = 3$, $p = 0.44$, Cramer's $V = 0.09$), and year-to-year differences in mislabeling percentages were not influenced by sampling effort (Spearman's $r = 0.8$, 2-tailed $p = 0.2$).

All sushi fish types, except bluefin tuna, were mislabeled at least once. Substitution rates, however, were not homogenous across fish groups (Fig. 2). All samples of halibut and red snapper were mislabeled (100%). Genetic identification of sushi samples showed that halibut was commonly substituted with flounder (*Paralichthys*

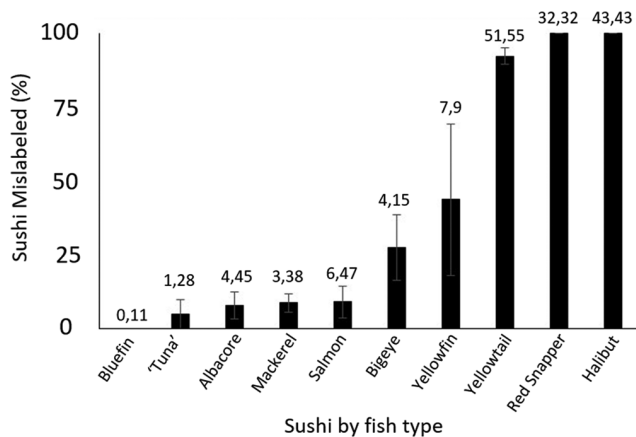


Figure 2. Mean (SE) frequency (%) of mislabeling of sushi fish from 2012 to 2015 (numbers above bars: total number of mislabeled samples, total number of samples).

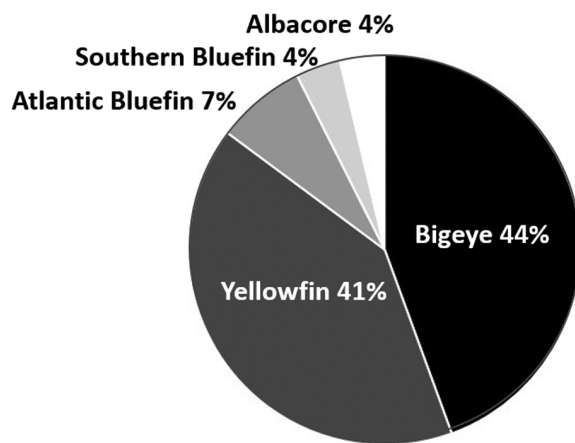


Figure 3. Percentages of sushi samples accurately sold as tuna, all of which are members of the genus *Thunnus*.

spp.). In total, 89% of red snapper samples (*Lutjanus campechanus*) were replaced by 8 different fish taxon, including red seabream (*Pagrus* spp.), 77% of the time. Substitution rate for yellowtail was also high (93%); 98% of the time it was substituted with amberjack (*Seriola quinqueradiata*). Substitution rates were lowest for salmon (13%) and mackerel (8%).

Mislabeling rates varied greatly in tuna. Bluefin tuna was never mislabeled, albacore tuna and the general tuna group had low rates of mislabeling (<10%), bigeye tuna had moderate rates (27%), and yellowfin tuna had very high rates (78%). Menu-labeled albacore, yellowfin, and bigeye tunas were often substituted with other tuna (80% of the time), but samples of the general tuna group were all but once an acceptable *Thunnus* species (Fig. 3).

Although sushi sample availability differed among fish groups ($n = 9\text{--}55$; Fig. 1), sampling effort did not explain variance in substitution rates among fish groups (Spearman's $r = 0.47$, 2-tailed $p = 0.17$). Furthermore, mislabeling rates within each fish type were not significantly associated across years (Fisher exact probability test, all test $p > 0.35$). However, when all tuna taxon were combined (albacore, bigeye, general tuna group, etc.) and analyzed among the 4 years, the association was significant (Fisher exact probability test, $p < 0.005$), which can be attributed to a high mislabeling rate in 2014 for only tuna groups.

Sushi Restaurants

All 26 sushi restaurants sampled had at least one incidence of species substitution; average mislabeling rate was 45.5%. A mean of 12.4 (SD 3.7) fish were sampled per restaurant, and a mean of 5.8 (SD 7.1) fish were mislabeled. Repeated mislabeling of the same type of fish was common in restaurants sampled in multiple years. A fish type mislabeled in 1 sampling year was mislabeled in at least 1 other sampling year 61% of the time. Furthermore, if mislabeling occurred in 1 year for a particular fish, mislabeling occurred every year that fish was sampled from that restaurant 92% of the time (33 of 37 samples).

Grocers

Fourteen of the 16 sampled fish filets from grocery stores were successfully amplified and identified to a known species on GenBank: 42% (6 of 14 samples) were mislabeled. Salmon ($n = 3$), albacore ($n = 2$), halibut ($n = 2$), and mackerel ($n = 1$) were all correctly labeled based on accepted market names. However, all yellowfin tuna ($n = 3$), red snapper ($n = 2$), and yellowtail ($n = 1$) were mislabeled and substituted with bigeye tuna, rockfish, and amberjack, respectively. All three sampled grocery stores had at least one case of species substitution.

Discussion

The DNA barcoding of fish sold in sushi restaurants showed a consistently high (>40%) substitution rate over the 4-year study period and stand in contrast to temporal changes in mislabeling rates that have been suggested, but not explicitly measured, in previous studies. For example, low mislabeling rates of 1.5–13% (Huxley-Jones et al. 2012; Helyar et al. 2014; Bénard-Capelle et al. 2015; Khaksar et al. 2015; Mariani et al. 2015) contrast with higher rates (>25%) reported in earlier studies (e.g., Wong & Hanner 2008; Logan et al. 2008; Warner et al. 2013) (Table 1). Authors of several of these articles attribute the declines to improved consumer awareness, increased mass media coverage on mislabeling, and new

food-labeling regulations. However, given that sampling methods, geographic regions, retailer types, fish-product type (fresh, frozen, or processed), and number of samples were not standardized among studies (Naaum et al. 2015), such comparisons across time should be interpreted cautiously.

Nearly half of all sushi samples were mislabeled, but not all fish were equally vulnerable to fraudulent practices; red snapper, halibut, yellowfin tuna, and yellowtail had the highest rates of mislabeling. Nationwide investigations of seafood fraud have similarly identified species vulnerable to mislabeling, and red snapper and halibut have consistently high substitution rates ($\geq 75\%$) (Wong & Hanner 2008; Khaksar et al. 2015).

Red Snapper

Warner et al. (2013) found that all snapper samples from the U.S. west coast were mislabeled, whereas samples from Miami, Florida, the location closest to the true range of snapper, had lower rates (38%). This dichotomy illustrates the importance of considering regional effects in the seafood supply chain when assessing species-specific mislabeling rates. For example, accurate identification in the Caribbean is complicated by the presence of overlapping, phenotypically similar species (*L. campechanus* and *L. purpureus*). Results of a population-genetics study of these species (Gomes et al. 2012) suggests they are not interchangeable and should continue to be managed as separate units. This necessitates accurate species-specific labeling to maximize sustainability of the red snapper fishery.

In California, where no true red snapper occur, the problem of mislabeling can largely be attributed to discrepancies in common name versus FDA approved naming conventions. In the past, Pacific red snapper was an acceptable name for 13 rockfish species (*Sebastes* spp.) (California Code of Regulations 1982). For many years, this ambiguous naming convention allowed for the lawful substitution of red snapper with multiple rockfish species. Two of the 13 legally permissible *Sebastes* species (*S. levis*, *S. ruberrimus*) were deemed overfished and closed to all fishing in California in the 1990s and remain closed to fishing in California today (CA Code of Regulations 2017), yet the original regulation (CA Code of Regulations 1982) was never amended. Hence, this legislation allowed for the substitution of one vulnerable, overfished species with another, demonstrating the larger conservation issues associated with regional loopholes and seafood mislabeling. Fortunately, recent changes to the California Fish and Game Code (section 2.8379, 2015) state that Pacific red snapper is no longer an acceptable market name for these rockfish species, bringing California labeling requirements in-line with the FDA (FDA CPG section 540.475 Snapper-Labeling). Although red snapper was

consistently mislabeled from year to year in our study, no red snapper sushi sample was genetically identified as *Sebastes*, but two grocery samples were, namely *Sebastes brevispinis* and *Sebastes goodei*.

Halibut

Mislabeling in halibut tends to occur at two levels: substitution of Atlantic halibut for Pacific halibut or vice versa and substitution of other flatfish, primarily flounders and hake, for products sold under the generic moniker *halibut* (Wong & Hanner 2008; Warner et al. 2013) or *Pacific halibut* (Warner et al. 2013). We found mislabeling of halibut was consistently high across sampling years; 89% of marketed halibut were identified as flounder (Fig. 2), a rate substantially higher than the 23% reported by Warner et al. (2013) and 67% reported by Wong and Hanner (2008). The 33% substitution rate of halibut with olive flounder (*P. olivaceae*) is a public health issue. Consumption of raw olive flounder, in which a myxospore parasite (*Kudoa septempunctata*) is present, caused extensive outbreaks of gastroenteritis in Japan (Kawai et al. 2012) (Matsukane et al. 2010; Iwashita et al. 2013). The 20% substitution rate with summer flounder (*P. dentatus*) is also a fisheries concern because the U.S. Atlantic fishery for summer flounder has been overfished and has exhibited declining biomass since 2010 (National Marine Fisheries Service [NMFS] 2015). The species is listed as a species to avoid on Seafood Watch (Seafood Watch 2016). Similarly, about 19% of halibut were substituted with southern flounder (*P. lethostigma*), a target of major fisheries in the Gulf of Mexico and offshore North Carolina. Fishery stocks offshore Texas and North Carolina have declined 25–30% over the past three generations and are listed as near threatened by the International Union for Conservation of Nature (IUCN) (Munroe 2015).

The high rate of halibut mislabeling may result from discrepancies between federal and California labeling laws. Per the FDA's Seafood List, halibut is the accepted market name for *Hippoglossus hippoglossus* and *H. stenolepis*, whereas California Fish and Game Code (section 8391, 2015) accepts *P. californicus*, commonly called California halibut. *Paralichthys californicus* is labeled as California flounder by the FDA. In our study, halibut sushi samples were not identified as *P. californicus*; rather, they were often substituted with southern, summer, and olive flounder, respectively.

Tunas

Tuna are one of the most highly fished and valuable stocks worldwide. Commercial tuna fisheries are tightly regulated by international commissions (e.g., Inter-American Tropical Tuna Commission and International Commission for the Conservation of Atlantic Tunas), which may

reduce fraud. However, our results showed a high rate of substitution in tuna. In Los Angeles fish sold as yellowfin tuna were almost always substituted with another tuna species, predominately bigeye tuna (58.3%). Yellowfin tuna were also sold as simply tuna, showing that the general tuna category is used to market a number of different species. The acceptance of a nonspecific common name like tuna as a suitable label makes comparisons of mislabeling rates across studies difficult and limits the ability to identify stocks of particular concern. Among our samples labeled as tuna, the majority were identified as bigeye or yellowfin tuna (Fig. 3), and only one was not an acceptable *Thunnus* species (*Seriola dumerili*). The IUCN categorizes bigeye tuna as vulnerable, and the eastern and western-central Pacific stocks are considered overexploited (Majkowski 2007; Collette et al. 2011a). Conversely, yellowfin tuna stocks are categorized as near threatened, and all stocks are being fished below maximum sustainable yield (Collette et al. 2011b). Hence, high rates of mislabeling of yellowfin tuna are even more problematic given that it is being substituted with a species of higher conservation concern. Two tuna samples were identified as Atlantic bluefin tuna and one as southern bluefin tuna, 2 species classified as endangered and critically endangered, respectively (Collette et al. 2011c; Collette et al. 2011d). Overall, grouping multiple species under a single common name poses significant barriers to understanding patterns of substitution critical to informing stock assessments and accurately aligning catch reports with consumer demand.

Yellowtail

We found high levels of mislabeling in yellowtail samples, a result in contrast to results of previous studies (Cawthorn et al. 2012; Khaksar et al. 2015). Our results may be due in part to our strict assignment of mislabeling based on FDA guidance, for which *Seriola lalandi* is the only accepted species for yellowtail, whereas other researchers also accepted *S. quinqueradiata* (amberjack) as a match (Cawthorn et al. 2012; Khaksar et al. 2015). In our study, substitution of one species of *Seriola* for another occurred in 48 of 51 mislabeled yellowtail samples. Furthermore, these species are differentiated in Japanese. *S. lalandi* is known as Hiramasa, and *S. quinqueradiata* is known as Hamachi or Buri. Both species are categorized as least concern (IUCN 2016). These 2 species along with *S. dumerili* (greater amberjack) comprise a major aquaculture fishery. *S. quinqueradiata* is cultured intensively and comprises roughly 80% of annual production worldwide (IUCN 2016).

Origins of Species Substitutions

Reducing seafood fraud to protect consumers and fisheries resources requires identifying where mislabeling

occurs. Seafood substitution was ubiquitous in the Los Angeles sushi restaurants we sampled. The same marketed fish were mislabeled in both sushi restaurants and high-end grocery stores, and that the same fish are commonly substituted year after year with no significant differences for a given species among years. These patterns could result from a concerted effort across retailers to mislead consumers. However, the more likely explanation is that mislabeling originates earlier in the supply chain, and retailers and consumers are victims of fraud. Although results of recent studies suggest mislabeling of seafood is declining, potentially due to new regulations and increased consumer awareness (Khaksar et al. 2015; Mariani et al. 2015), the consistently high rate of mislabeling we observed indicates otherwise and suggests targeting early points in the supply chain will likely have the biggest impact on reducing mislabeling.

Conservation Implications

Although we documented consistently high rates of seafood mislabeling, the species commonly used as substitutes were often of lower conservation concern than the taxa offered by the vendor. For example, red snapper, categorized as vulnerable by the ICUN largely due to extensive recreational and commercial fishing pressure, was most commonly replaced with sea bream, which is categorized as least concern (Russell et al. 2014; Anderson et al. 2015). Similarly, the conservation status of olive flounder is of lower priority than halibut; halibut was commonly substituted with olive flounder. These findings align with an assessment of multiple mislabeling studies by Stawitz et al. (2016), who found that mislabeling generally leads to the sale of species of lower conservation concern.

Although this general trend suggests mislabeling may inadvertently supply more sustainable seafood to consumers, the implications are disparate across taxa (Stawitz et al. 2016). For example, bigeye tuna, classified as vulnerable, was a common replacement for other tuna species, particularly yellowfin tuna which is classified as near threatened in part due to more effective regulations (Collette et al. 2011a, 2011b). Therefore, although seafood mislabeling remains an egregious offense, from a sustainability standpoint this information can be used to prioritize mitigation efforts aimed at species of concern and their substitutes.

Policy Implications

There are ongoing efforts by the United States and other nations to combat the global problems of illegal, unreported, and unregulated fishing. However, as policies are developed, it is important to consider which agencies will be responsible for monitoring and compliance with seafood-labeling regulations. In Los Angeles, the

County Department of Public Health's Bureau of District Surveillance and Enforcement (DSE) is responsible for compliance (Bureau of DSE 2016). The DSE enforces the California Retail Food Code and Title 21 of U.S. Code of Federal Regulations, both statutes requiring operators to use acceptable market names recognized by the FDA (Bureau of DSE 2016). The DSE also conducts truth-in-menu investigations at restaurants to validate labeling of food sold, a common tool in the United States (Thomas & Mills 2006). Current monitoring methods by DSE are limited to visual inspection of products and reviewing shipment invoices for inconsistencies; the burden of proof is on the operator. Our results indicate these methods are failing and that DNA barcoding would be an effective alternative for ensuring compliance with proper-labeling regulations.

Rising global demands for seafood are driving the proliferation of illegal fishing practices, including mislabeling and illegal substitution of seafood. The United States imports an estimated 20–32% of the global fish supply by weight, about \$2.1 billion of the U.S. fish market (Pramod et al. 2014). Therefore, the United States should be setting a global example by raising awareness and marketing sustainable seafood choices with certification tools and ranking schemes such as Seafood Watch and the Marine Stewardship Council (though these certifications are not without concerns; see Christian et al. 2013). Critical to addressing seafood mislabeling is effective monitoring of and compliance with existing international enforcement measures (e.g., FAO Amendment on Port State Measures on IUU, 2015) and new regulations pending in multinational trade agreements (e.g. TPP environmental provisions, 2016) and U.S. federal programs (e.g. Commerce Trusted Trader Program, Billing code 3510-22-P, 2016). However, the effectiveness of federal regulations and consumer choice to shape sustainable fisheries relies on accurate seafood labeling. Further study of all levels of the supply chain will be critical to determine the origins of seafood fraud and increase seafood-labeling accuracy.

Recommendations

Addressing the global challenge of seafood mislabeling requires complementary actions at all stages of the seafood supply chain. To meet this challenge, we recommend the following measures. Develop and support international and federal policy that strengthens traceability in seafood products through the clear labeling of the country of origin, scientific name, wild-caught versus farmed-raised fish, and use of environmentally conscious fishing practices (e.g., ecolabels, Uchida et al. 2014). Increase enforcement of existing policies that require accurate labeling of food (e.g., section 4205 Nutrition Labeling Provision under the U.S. Affordable Care Act; section 113729.5 Acceptable Market Name under the California Retail Food Code). Build

the monitoring capacity of inspectors to identify seafood labeling inconsistencies through increased training, and incorporate emerging technology such as portable, hand-held DNA sequencers (Hayden 2015). Use monitoring to inform retailers when they are victims of species substitutions, allowing them to pressure wholesalers and fishers to increase reliability of labeling throughout the supply chain. Finally, promote the democratization of DNA barcoding by consumers through education and the use of citizen science (Adamowicz & Steinke 2015), and increase public awareness through the use of social media and crowd-sourced consumer review mobile applications (e.g., Yelp).

Seafood Monitoring as Education

One of the earliest studies in which genetic methods were used to assess the frequency of seafood fraud came from an undergraduate science laboratory class (Marko et al. 2004), a model employed in subsequent studies (e.g., Cline 2012; Naaum & Hanner 2015), including this one. The value of this model is two-fold. First, it provides a way to conduct longitudinal studies that may prove otherwise difficult and provides valuable insight into whether stricter regulations result in less seafood fraud. A second powerful outcome was the impact it had on students. Many students were unaware of this problem and were shocked that seafood mislabeling was so common. By witnessing the problem firsthand, they could become powerful voices in discussions of seafood fraud, thereby greatly expanding awareness of this important conservation issue.

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