Food Control 181 (2026) 111733

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

DNA barcoding and metabarcoding reveal labeling accuracy and species substitution in squid and surimi from Los Angeles markets

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ARTICLE INFO

Keywords:
DNA barcoding
DNA metabarcoding
Mislabeling
Seafood
Surimi

ABSTRACT

Accurate species identification of seafood remains a persistent challenge in local and global marketplaces. This is particularly true for processed products that obscure diagnostic features. Here, we conduct a dual-molecular investigation into frozen squid and imitation crab products sold at American, Asian, and Hispanic grocery stores in Los Angeles, California, to assess label integrity under current U.S. Food & Drug Administration (FDA) guidance. Forty-eight squid samples from 13 retailers underwent DNA barcoding and yielded high percentage matches to 10 species across six genera. Despite broad labeling as 'squid', all samples matched FDA Acceptable Market Names with a 0 % mislabeling rate. In contrast, DNA metabarcoding of 43 imitation crab products revealed an average of six detected species per sample, with only 72 % of samples containing at least one package listed seafood ingredient. *Gadus chalcogrammus* (Alaska pollock) and *Doryteuthis gahi* (Patagonian squid) were the most frequently detected species, with the former often included as a listed ingredient and the latter never listed. 95 % of imitation crab products contained at least one undeclared species, including detection of endangered shark species and squid species prone to illegal fishing. Our results illustrate a high labeling fidelity for single species packaged squid, yet a discrepancy between legal labeling allowances and actual product composition for blended species surimi. Based on our findings, we encourage refining and the addition of routine DNA-based surveillance of blended seafood products to improve labeling credibility, consumer confidence, and supply chain integrity.

Introduction

Species mislabeling is a complex and persistent challenge that touches all levels of the global seafood supply chain, from importers and processors (Senathipathi et al. 2024; Shehata et al. 2019; Willette et al. 2021) to grocery stores and restaurants (Khaksar et al., 2015; Pardo et al. 2016; Willette et al. 2017). Mislabeling incidences are generally lower earlier in the supply chain (*e.g.* at harvest or processing) with the highest rates reported at end of the supply chain (*e.g.*, in restaurants) (Shehata et al. 2019; Willette et al. 2021). Progress in reducing mislabeling has been successful through intervention and information sharing. For example, seafood mislabeling rates in traditional European markets fell from 25 % to 5 % after reporting requirements improved for processors (Mariani et al. 2015), mislabeling rates for cod sold in Ireland supermarkets dropped from 34 % to 0 % after robust media attention increased public awareness (Mariani et al. 2015), and sushi mislabeling rates declined from 44 % to 15 % after Los Angeles restaurants that joined a local seafood monitoring campaign (Willette et al. 2025). These and other like studies suggest seafood labeling accuracy can be substantially improved with the use of available tools and best practices.

Seafood is mislabeled when it is marketed under a name that misrepresents its actual taxonomic identity, a fraudulent act in the United States (U.S.) (21 U.S.C. Ch. 9 § 403), the European Union (E.U.) (E.U. Regulation No. 1379/2013), China ((China General Order No. 123 of 2009) Australia (Australia Consumer Law Sch. 2 CCA 2010), and the Philippines (Philippines Republic Act no. 7394), as well as in many other jurisdictions around the world. Understanding why mislabeling occurs and appreciating why mislabeling matters are both important components of addressing this challenge. Mislabeling can arise when differences in the required reporting of key data elements, such examples include species name, product name, or geographic origin, or the lack of such reporting, are not consistently adhered to throughout the supply chain. Likewise, broad naming conventions or the lack of precise species-level labeling requirements can result in labeling errors. For

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https://doi.org/10.1016/j.foodcont.2025.111733
Received 3 July 2025; Received in revised form 16 September 2025; Accepted 17 September 2025
Available online 19 September 2025
0956-7135/© 2025 Published by Elsevier Ltd.

example, the U.S. Food and Drug Administration's Seafood List which provides the Acceptable Market Name and corresponding scientific name for seafood products sold in the U.S., lists 'squid' as the single Acceptable Market Name for at least 29 cephalopod species from the common genera of *Doryteuthis, Illex, Loligo, and Septioteuthis* (U.S. Food and Drug Administration Seafood List, 2025). Seafood mislabeling matters because it can levy economic costs on customers, inflict allergy or toxin health impacts on consumers, and deceive consumers of their dietary choices for religious or environmental reasons (Giusti et al. 2024). Mislabeled seafood that is sourced from illegal fishing or from unregulated waters can also indirectly facilitate human rights violations (Brito & Willette, 2023; Mendoza et al., 2016).

Some types and forms of seafood are more vulnerable to mislabeling and fraud than others. For example, processed seafood products are often stripped of their diagnostic features making morphological identification impossible. Examples include fish cakes, fish balls, and minced fish sticks. Indeed, transforming the appearance of one type of seafood to look like another is the intention of certain processed seafood such as surimi. Surimi, which translates to 'grounded meat' in Japanese, is a processed seafood paste made from grounded, white-fleshed fish like Alaskan pollock, Pacific whiting, and threadfin breams, and formed into imitation crab sticks and flakes (Leadbitter et al. 2020, FDA CPG Sec. 540.700). A growing literature investigating the ingredient composition of surimi indicates this highly processed seafood product is also vulnerable to mislabeling, with rates over 80 % (Hu et al. 2025).

DNA barcoding is a potent and frequently applied molecular genetics method to investigate and validate the taxonomic identification of fresh and frozen seafood. This method is frequently applied at the end of the supply chain (*e.g.*, restaurants, fish markets, grocers), and has been demonstrated robust and effective in seafood identification studies in over 35 countries (Willette et al. 2025). DNA barcoding typically leverages Sanger sequencing, a highly accurate first-generation sequencing technique that generates long DNA (>500 nucleotide base pairs (bp)) with a very low error rate (Shendure & Ji, 2008). The longer the DNA sequence, the higher the resolution for differentiating between genetically similar species. Two limitations of DNA barcoding, however, are its low-throughput rate in producing reads, and that only one species can typically be detected at a time, generally the species' DNA most abundant in a sample (Cristescu, 2014). These limitations can be overcome with the combination of next generation sequencing and DNA barcoding, known as DNA metabarcoding. Next generation sequencing utilizes large-scale parallel sequencing to produce orders of magnitude more sequences than Sanger sequencing, including amplification of low frequency DNA molecules (Medinger et al. 2010). This enables the detection of multiple species from a single sample, including when a sample may only contain partial tissue and cells from each species (Deiner et al. 2017; Kelly et al. 2014). Thus, the complementary attributes of highly accurate DNA barcoding with the multi-species detection capacity of DNA metabarcoding can be leveraged together to broaden surveillance of seafood labeling accuracy and help combat fraud (Andronache et al. 2025).

Los Angeles, California, with its diverse population and wide array of seafood retailers, offers a strategic urban context to assess the labeling accuracy of squid and surimi products using molecular tools. This study aims to detect and quantify the incidence of seafood mislabeling of frozen squid (in multiple forms including rings, chunks, and battered calamari) and imitation crab products from American, Asian, and Hispanic-food grocers in Los Angeles. The findings will fill a persistent data gap in scientifically verified assurance that package labeling matches product contents, and will reveal, if any, the presence of other included species. This study will use these findings to evaluate patterns and trends in labeling of these products and discuss potential regulatory and ecological implications.

Methods

Sampling

Fifty (50) squid products were purchased between 07 July and August 12, 2024 from thirteen (13) different grocers in Los Angeles, California. Seven samples were from Hispanic grocers, 10 from American grocers, and 33 from Asian grocers. Sample information including processing (pre-cooked, raw, breaded), form (frozen, canned, etc.), name of 'Product of' country, scientific name of squid species (if listed), and other metadata were recorded (see Supplemental Materials S1). The brand name, product name, and U.S. importer or parent company name data was also recorded but is not disclosed in this study as the purpose of this work is not punitive but rather is to support transparency in the seafood trade. Photographs were taken of the front and back of each package and of the sales receipt, when possible. A corn-kernel-size piece of squid meat was sub-sampled and stored in a 2 mL tube with 80 % ethanol for downstream processing and sequencing which was completed in August 2024. Likewise, 48 imitation crab meat products were purchased between 20 November and December 16, 2024 from nineteen (19) different grocers in Los Angeles, California. Five samples were from Hispanic grocers, 13 from American grocers, and 30 from Asian grocers. Eleven of the grocers were the same as the squid and imitation crab sampling, with two and eight being unique, respectively. Metadata, including ingredient list, and 'Product of' country, were recorded (see Supplemental Materials S2), with photographs taken of the front and back of each package and of the sales receipt, when possible. The brand name and importer/parent company were also noted yet is not disclosed here. Similarly, a corn-kernel size piece of imitation crab was preserved in 80 % ethanol in a 2 mL tube until downstream processing and sequencing which was completed between April and June 2025. We took care to avoid cross-contamination between the squid DNA barcoding and surimi DNA metabarcoding portions of this project, including sterile lab techniques and months-long separation of work.

DNA barcoding and data analysis

Approximately 25 mg of each preserved sample was extracted for genomic DNA using the Zymo Research (San Diego, CA) Quick-DNA miniprep plus kit following manufacturer's instructions for blood and tissue samples. A 650-bp fragment of the mitochondrial COI gene region was amplified using the primers HCO (5′ TAAACTTCAGGGTGACCAAAAAATCA 3′) and LCO (5′ GGTCAACAAATCATAAAGATATTGG 3′) (Folmer et al., 1994), and Cytiva puReTaq Ready-To-Go polymerase chain reaction (PCR) bead 0.2 mL tubes (Marlborough, MA). The thermocycle protocol included an initial denaturing step at 94 °C for 5 min, then 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. PCR product visualized using gel electrophoresis. Successful PCR product was purified and sequenced in both directions at a commercial sequencing facility (Psomagen, Rockville, MD). Species-level identification of resulting FASTA sequences was performed using the National Center for Biotechnology Information's (NCBI) Basic Local Alignment Search Tool (BLASTn) using the MegaBLAST algorithm (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Species-level identification was determined for sequences with a ≥97 % match or higher. This DNA-based identification was then contrasted to the product name listed on the corresponding package. Squid was deemed mislabeled if the product name did not match the Acceptable Market Name as specified by the FDA Seafood List (US FDA CFR 101.18c). Mislabeling rates were averaged by grocer type and compared using the Agresti-Caffo test (Agresti & Caffo, 2000). DNA barcoding is a highly accurate, well-vetted method, we acknowledge this method inherently involves two types of uncertainty. The first is our reliance on the accuracy and completeness of the publicly available reference database, which may contain gaps or errors.

The second is potential error during the PCR amplification, yet today's high-fidelity polymerases exhibit a very low error rate (below 1 %), thus it is unlikely to significantly impact species identification given our ~650-bp fragment length.

DNA metabarcoding and data analysis

Genomic DNA was extracted from approximately 25 mg of imitation crab meat using the above Quick-DNA miniprep extraction kit and manufacturer's instructions. The gDNA then used to amplify the COI gene region (~670-bp fragment) through a first PCR. We used the HCO and IntF (5′ GGWACWGGWTGAACWGTWTAYCCYCC 3′) primers (Leray et al. 2013) modified to include the Oxford Nanopore Technology (Oxford, UK) (ONT)-specific primer extensions (FW-extension 5′ TTTCTGTTGGTGCTGATATTGC 3′ and RV-extension 5′ ACTTGCCTGTCGCTCTATCTTC 3′) compatible for downstream sequencing. We used a touchdown protocol to increase binding targets of the primers. This thermocycle protocol included an initial denaturing step at 95 °C for 3 min, then 17 cycles of denaturing at 95 °C for 10 s, annealing at 62 °C for 30 s then reduced by 1° at each subsequent cycle, and extension at 72 °C for 60 s. This was followed by 26 additional cycles of denaturing at 95 °C for 10 s, annealing at 46 °C for 30 s, and extension at 72 °C for 60 s with a final extension at 72 °C for 10 min. PCR product was purified using AMPure XP beads (Agencourt, Beverly, MA) and quantified using the Promega QuantFlor ONE dsDNA system and fluorometer (Madison, WI), both following manufacturer's instructions. We found concentrations of at least 1.7 ng/μL were necessary for subsequent processing. A second PCR was then performed to add a unique barcode to each sample, using the ONT PCR barcoding expansion 1–12 kit, followed by purification using the AMPure XP beads (reduced from a 1.8x to 1x ratio to 2nd PCR product volume), then quantified. Up to 12 barcoded samples were then pooled equimolarly to a total concentration of 1 μg. The pooled DNA then underwent an enzymatic repair and end-prep step using the NEB Next FFPE DNA repair kit and NEB Next Ultra II end-repair module (New England Biolabs, Ipswich, MA), again following manufacturer's instructions. The resulting product was purified again using AMPure XP beads at the 1x proportion, followed by adapter ligation using the ONT Ligation Sequencing Kit v14, and a final bead purification step to obtain a high-quality sequencing library. Our lab used the ONT R10.4.1 flow cell which requires a concentration of 5–50 *f* mol. The library was loaded into a primed flow cell on the ONT MinION Mk1C device and sequenced via the MinKNOW interface selecting the above used ligation sequencing kit, barcoding expansion kit, and quality score of Q7.

Generated FASTQ output files were processed using our lab's custom-built Python Flet-supported desktop application for genomic data called the Genomic Analytics Tool (GNAT; Available on GitHub, https://github.com/Intechgambit/giat). GNAT functions by converting the sequence output format FASTQ files to FASTA files which are then searchable by the NCBI BLAST tool. In GNAT we curated an NCBI BLAST-searchable database of 50 target species of interest (see Supplemental Materials S3). Converted FASTA files were then searched against this database to assign taxonomy. These species were curated from squid species detected in the DNA barcoding portion of this study, from the listed ingredients on imitation crab packages, and from Lee et al. (2024), a recent study that also investigated the contents of surimi products. The taxonomic assignments were then annotated and saved into CSV format. Sequences with a ≥97 % percent identity match were carried forward in the analysis. These data were summarized into descriptive statistics, and taxonomic assignments were listed for each sample as a cumulative percentage. Taxa representing at least 1.0 % of high-quality species-level matches (cut-off threshold) of a given sample were reported. Lastly, we cross-referenced each sample's ingredient list against the DNA taxonomic assignments. All but one sample included at least one FDA Acceptable Market Name in the ingredient list. Our results report a 'detection rate', the frequency at which samples contained at least one listed Acceptable Market Name ingredient and report a 'detection rate' of products with species not included on the ingredient list.

Results

Squid DNA barcoding

The 50 squid products were sold under 31 brands from 28 importers/parent companies, of which 78 % (22/28) were based in California. Of the 50 products, 44 (88 %) were frozen, and 6 (12 %) were canned. Countries listed as 'Product of' included China (48 %), Spain (12 %), Korea (8 %), Thailand (6 %), United States (6 %), Peru (4 %), and France, India, Italy, New Zealand, Taiwan, & Vietnam (2 % each). Two packages (4 %) had no 'Product of' labeling. Product names on packages were numerous and included examples such as 'squid rings'; 'frozen squid'; 'squid pieces'; jumbo calamari'; 'calamari tubes'; seafood mix'; 'and 'seafood blend'. The package ingredient list was narrower, with 43 listed as 'squid' (or some specific portion of squid such as tentacles, cuts, etc.), six listed as 'calamari', and one with no ingredient listed. Only six of the 50 products included the scientific name for the contents.

Successful DNA sequences were obtained from 48 of the 50 squid samples, with the two samples of canned squid 'in ink sauce' unsuccessful. The mislabeling rate of the remaining samples was 0 % (0/48) per U.S. FDA Seafood List naming conventions. The mislabeling rate was 0 % for all samples from each type of grocer, and thus the Agresti-Caffo test could not be performed as there was no difference. One sample for which a scientific name was listed, *Uroteuthis chinensis*, did not match the DNA-based taxonomic assignment of *Doryteuthis gahi*. All samples had a 99 %–100 % match to the NCBI BLASTn species results (Supplemental Materials S1), inferring a very high level of species identification.

Ten distinct species of squid were identified (Table 1). *Dosidicus gigas* (Humboldt squid) was the most identified squid species (35 %), followed by *Illex argentinus* (Argentine shortfin squid) (30 %) and *Uroteuthis duvaucelii* (Indian squid) (13 %). *Doryteuthis gahi* (Patagonian squid), *Doryteuthis pealeii* (Longfin inshore squid), *Todarodes pacificus* (Japanese flying squid), and *U. chinensis* (Mitre squid) each represented 4 %, and *Doryteuthis opalescens* (Opalescent inshore squid), *Sthenoteuthis oualaniensis* (Purpleback squid), *and U. edulis* (Swordtip squid) were 2 % each (Fig. 1).

Of the 10 species identified, 'Product of China' was listed as a source for 7 species, and exclusively for the only samples identified as *S. oualaniensis*, *T. pacificus*, and *U. edulis*. Likewise, two species, *Doryteuthis opalescens* and *D. pealeii* were exclusively listed as 'Product of United States'.

Imitation crab DNA metabarcoding

The 48 imitation crab meat products were sold under 29 brands from 26 importers/parent companies, of which 38 % (18/48) were based in California. All 48 products were labeled as fully cooked and sold frozen. Countries listed as 'Product of' include Korea (23 %), United States (21

Table 1
Scientific and common name of detected squid species based on DNA sequencing, as well as the frequency and percentage of the study's 48 samples.

Scientific name	Common name	Number (%)
Doryteuthis gahi	Patagonian squid	2 (4 %)
Doryteuthis opalescens	Opalescent inshore squid	1 (2 %)
Doryteuthis pealeii	Longfin inshore squid	2 (4 %)
Dosidicus gigas	Humboldt squid	17 (35 %)
Illex argentinus	Argentine shortfin squid	14 (29 %)
Sthenoteuthis oualaniensis	Purpleback squid	1 (2 %)
Todarodes pacificus	Japanese flying squid	2 (4 %)
Uroteuthis chinensis	Mitre squid	2 (4 %)
Uroteuthis duvauceli	Indian squid	6 (12 %)
Uroteuthis edulis	Swordtip squid	1 (4 %)

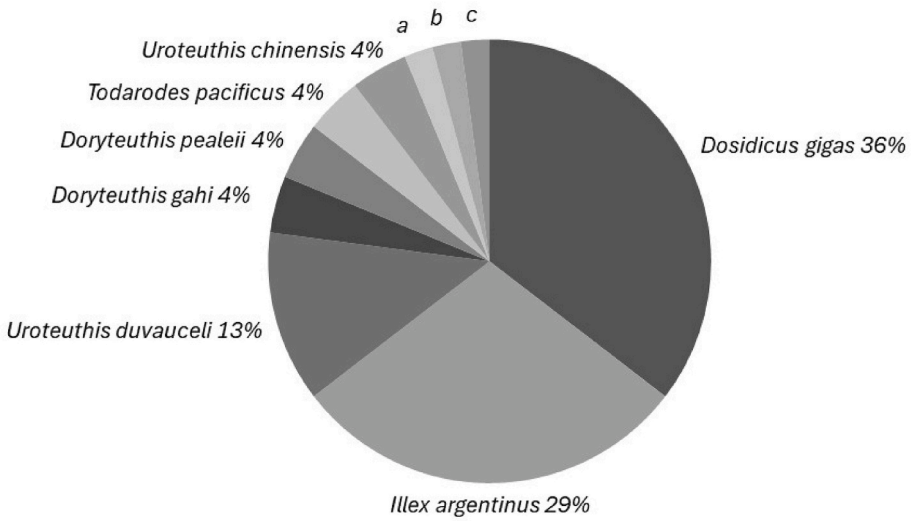

Fig. 1. Squid samples DNA-based identification by species. Letter 'a' represents *Doryteuthis opalescens,* 'b' represents *Sthenoteuthis oualaniensis*, and 'c' represents *Uroteuthis edulis*, each at 2 % of samples.

%), Thailand (17 %), China (15 %), India (10 %), Japan (2 %), and Spain (2 %), with five products with no label (10 %). A total of 27 different organisms were specified on the ingredient labels of the 48 packages, including 15 fish, seven crabs, four lobsters, and one eel (see Supplemental Materials S2). Alaska pollock was the most common listed fish ingredient (75 % of samples), followed by threadfin bream (45 %) and Pacific whiting (33 %). Crab meat was listed as an ingredient for 35 % and crab extract listed for 60 % of samples. We found this an interesting result, as we did not expect products labeled and marketed as imitation crab meat to contain actual crab meat.

A total of 28, 234, 221 post-filtered reads were obtained from 43 successful imitation crab meat samples, with an average of 91,670 reads per sample. Five samples were not successful in sequencing, potentially due to the quality, processing, or condition of the samples purchased.

A total of 38 unique species were detected from the curated database across all species. The average number of species detected using DNA metabarcoding was 6.4 (SD ± 3.1), whereas the average number of listed seafood ingredients (*e.g.,* pollock, whiting, etc.) per sample was 3.1 (SD ± 1.3) (Supplemental Materials S2). *Gadus chalcogrammus* (Alaska pollock) was the most frequently detected fish species, found in 67 % of all samples (Table 2). Other common fish species included *Alopias pelagicus* (pelagic thresher, 63 %), *Carcharhinus obscurus* (dusky shark, 35 %), *Megalops cyprinoides* (Indo-Pacific tarpon, 28 %), *Trichiurus lepturus* (Atlantic cutlassfish, 16 %), *Merluccius productus* (North Pacific hake, 14 %), and *Nepipterus randalli* (Randall's threadfin bream, 14 %). *Doryteuthis gahi* (Patagonian squid) was the most common invertebrate species detected, also found in 67 % of all samples. Other common invertebrates included *U. duvaucelii* (63 %), *T. pacificus* (60 %), *S. oualaniensis* (60 %), and *D. pealeii* (14 %). The remaining 26 taxa were each detected in less than 10 % of the time (3 or fewer samples). Squid were detected in 30 samples (70 %) and, for products sold as imitation crab, the presence of three crab species were detected in at least one sample, *Chionoecetes japonicus* (red snow crab), *C. opilio* (snow crab), and *Callinectus sapidus* (blue swimming crab). Our curated database included eight cartilaginous species detected by Lee et al. (2024) in their study of surimi products in Taiwan (Supplemental Materials S1). We detected four of the eight species Lee et al. (2024) detected, with at least one shark species detected in 62 % (24/43) of our study's samples.

Lastly, we report a detection rate of at least one listed Acceptable Market Name ingredient as 72 % (31/43 samples), which would equal a 28 % mislabeling rate (Fig. 2). Furthermore, 95 % (41/43) of samples were found to contain DNA of at least one additional species not included on the ingredient list.

Discussion

Squid product labeling accuracy

Our study found no mislabeling of squid when contrasting the DNA-based species identification against the US FDA Acceptable Market Name. This 0 % mislabeling rate was consistent across the sampled Asian, American, and Hispanic grocers. This is a sharp contrast from previous DNA-based testing of seafood sold from grocers in Los Angeles, which Willette et al. (2021) found an 11.4 % mislabeling rate for various fish purchased from 13 different grocers. Their study found most mislabeling was the result of substitution among species of the same genus (*i.e.,* Atlantic halibut sold as Pacific halibut) or sold under an invalid FDA name (*i.e.,* 'true cod', 'black cod'). A notable methodology difference between these studies is that all samples by Willette et al. (2021) were purchased as fresh cuts from the grocer's seafood counter and all were labeled Marine Stewardship Council (MSC) certified fish, whereas all squid in this study were purchased as frozen, pre-packaged products. DNA-based species identification studies of cephalopods in the US are severely lacking and warrant attention in addition to this study in Los Angeles. Beyond the US, other studies testing frozen products include Fernandez et al. (2020) who tested 25 frozen squid and octopus purchased from 3 grocers in northern Spain yet found a higher mislabeling rate of 31 %, whereas Paiva et al. (2024) included 53 processed squid ring samples from supermarkets in Brazil and French Guiana concluded 70 % were inaccurately labeled. Importantly, Paiva et al. (2024) state that although 37 of the 53 samples were inaccurately labeled, there were no instances of fraud as all samples were labeled as some squid product (lula nacional/national squid, lula em anéis/squid rings, or calmars marinés/marinated squid). The 70 % mislabeling rate is a reflection of Brazilian labeling policy that requires species name be included on the packaging. In the current study, we identified 10 unique squid species, yet US FDA guidance permits all 10 to be labeled as squid with no further requirement for species names. Thus, current US labeling policy permits a broad classification of species to be labeled as 'squid' (US FDA SeaFood and Agriculture Organization of the United Nations, 2025), that is not accepted in other marketplaces, nor for other seafood products in the U. S., such as Pacific halibut or Atlantic cod (see Willette et al., 2021). To address this challenge, we recommend the US FDA's Center for Food Safety and Applied Nutrition take similar action to how ambiguity was resolved among five *Seriola* fish that previously only allowed 'Amberjack' as the Acceptable Market Name yet were revised to include distinct Common Names for improved labeling (US FDA CFR 101.18c, Willette

Table 2
Ranked frequency of the 38 detected species from the 43 imitation crab (surimi) samples based on DNA metabarcoding. The scientific name, common name, the number and percentage of the 43 samples containing the given species. ** notates a critically endangered species, * notates an endangered species, # notates a CITES Appendix II listed species, and ^ notates a species of potential health risk.

Rank	Scientific name	Common name	# of samples	% of samples
1	*Gadus chalcogrammus*	Alaska pollock	29	67 %
	Doryteuthis gahi	Patagonian squid	29	67 %
2	*Uroteuthis duvaucelii*	Indian squid	27	63 %
	Alopias pelagicus#*	pelagic thresher	27	63 %
3	*Todarodes pacificus^*	Japanese flying squid	26	60 %
	Sthenoteuthis oualaniensis	purpleback squid	26	60 %
4	*Carcharhinus obscurus**	dusky shark	15	35 %
5	*Megalops cyprinoides*	Indo-Pacific tarpon	12	28 %
6	*Trichiurus lepturus*	Atlantic cutlassfish	7	16 %
7	*Merluccius productus*	North Pacific hake	6	14 %
	Nemipterus randalli	Randall's threadfin bream	6	14 %
	Doryteuthis pealeii	longfin inshore squid	6	14 %
8	*Carcharhinus sorrah*	spottail shark	4	9 %
	Carcharhinus falciformis	silky shark	4	9 %
9	*Nemipterus japonicus*	Japanese threadfin bream	3	7 %
	Nemipterus furcosus	fork-tailed threadfin bream	3	7 %
	Saurida isarankurai	shortjaw saury	3	7 %
	Nemipterus tambuloides	fivelined threadfin bream	3	7 %
	Upeneus japonicus	Bensasi goatfish	3	7 %
	Chionoecetes japonicus	red snow crab	3	7 %
	Taractes rubescens	pomfret	3	7 %
10	*Mulloidichthys vanicolensis*	yellowfin goatfish	2	5 %
	Nemipterus nematophorus	doublewhip threadfin bream	2	5 %
	Chionoecetes opilio	snow crab	2	5 %
11	*Pesudupeneus maculatus*	spotted goatfish	1	2 %
	Mulloidichthys vanicolensis	yellowfin goatfish	1	2 %
	*Anguilla**#	European eel	1	2 %
	Saurida lessepsianus	Lessepsian lizardfish	1	2 %
	Coryphaena hippurus	common dolphinfish	1	2 %
	Synodus variegatus	varegated lizardfish	1	2 %
	Engraulis ringens	Peruvian anchoveta	1	2 %
	Nemipterus bathybius	yellowbelly threadfin bream	1	2 %
	Nemipterus aurora	Dawn threadfin bream	1	2 %
	*Sphyrna lewini**	scalloped hammerhead	1	2 %
	Lobothorax typus	Isopod	1	2 %
	Callinectes sapidus	blue swimming crab	1	2 %
	Pseudupeneus maculatus	spotted goatfish	1	2 %
	Nemipterus hexodon	ornate threadfin bream	1	2 %

et al. 2018).

DNA metabarcoding of surimi/imitation crab

Our results substantiate DNA metabarcoding as a viable tool to simultaneously detect multiple species from mixed seafood surimi products. Findings from this Los Angeles-centered study are consistent with recent studies applying DNA metabarcoding specifically to surimi, including in Europe (Giusti, Armani, & Sotelo, 2017, Andronache et al. 2025) and Asia (Hu et al. 2025; Lee et al. 2024). Hundreds of species of seafood are known to be used in the production of surimi (Leadbitter et al. 2020), including at least 80 species of fish and cephalopods (Giusti, Tinacci, et al., 2017), Giusti, Tinacci, et al., 2017nd 35 shark species (Lee et al. 2024). Our study detected an average of 6.4 species per surimi sample, which is consistent with Zhang et al. (2024) and Giusti, Tinacci, et al. (2017) that detected ~5 species per sample, yet less than the 40 species per sample by Lee et al. (2024). Likewise, the most common fish (*G. chalcogrammus*) we detected is often the most common fish used and detected in surimi products (Giusti, Tinacci, et al., 2017; Leadbitter et al. 2020). Our study's most common invertebrate, *D. gahi* (Patagonian squid), was found in 67 % of surimi samples, but never included on any ingredient list (Supplemental Materials S2) and not reported in past metabarcoding results (Giusti et al. 2017; Lee et al. 2024, Zhang et al. 2024). Thus, future studies should be attentive to the possible presence of squid being incorporated, yet not declared, in processed seafood products.

Regulatory implications

This study's aim to analyze squid using DNA barcoding and surimi using DNA metabarcoding reveal broad, yet technically accurate labeling under current US FDA guidance. These findings do, however, highlight an existing gap between legal labeling allowances and the specific species present in commonly consumed products of squid rings, calamari, and imitation crab sticks & flakes sold in Los Angeles' American, Asian, and Hispanic grocery stores. A primary function of US FDA regulations is to ensure all US sold seafood products are wholesome and safe for human consumption (HACCP Regulation 21 CFR Part 123). Although squid does present a generally low risk to heavy metal exposure (*e.g.,* mercury), most species of squid are considered safe to eat when consumed in moderation (Torrinha et al. 2014). An exception is *T. pacificus* (Japanese flying squid), which can produce tropomyosin that may cause severe allergic reactions in sensitive individuals (Miyazawa et al. 1996). *Todarodes pacificus* was detected in 4 % of squid samples and 60 % of surimi samples. 'Squid' is the Acceptable Market Name for *T. pacificus*, like at least 28 other species, however, greater specificity for species that may present health risk should be updated given the increasing capacity to detect such species using DNA-based tools. Stronger US FDA guidance could be modeled from existing approaches including Spain's List of Trade Names database (MAPA 2019) that provides specific common names for different species by region, an actionable solution that improves both traceability and consumer confidence in which seafood is being sold.

In the US, labeling guidance for blended seafood products, including surimi, is mandated through CPG Sec. 540.700. Although not legally enforceable alone, this guidance requires blended fish protein products to be labeled as 'imitation', and to list all ingredients of the finished product. A limitation of this guidance for combatting mislabeling is that it does not require disclosure of all species used, but rather the term 'fish protein' followed with a parenthetical listing of the Acceptable Market Names (US FDA CFR 101.18c) of seafood that may be present. The current guidance may leave space that masks the true identity of the seafood products, including endangered species, overfished species, and species that may contain allergens. Indeed, this study detected the critically endangered *Anguilla* (European eel) and *Sphyrna lewini* (scalloped hammerhead shark), endangered *Carcharhinus obscurus* (dusky shark), *Alopias pelagicus* (pelagic thresher shark), *Isurus oxyrinchus* and *A. japonica* (Japanese eel), none of which were included in the ingredient list of sampled products. *Anguilla Alopias pelagicus, I. oxyrinchus, S. lewini*, and several *Carcharhinus* species detected in our study are also listed on the CITES Appendix II, thus are species that may require export permits (Convention on International Trade in Endangered Species of Wild Fauna and Flora, n.d.). Many of these species are listed on the CITES Appendix II, and thus are species protected from international trade. US guidance on where a product comes from, the 'Product of' information, falls under the US Country of Origin Labeling (COOL)

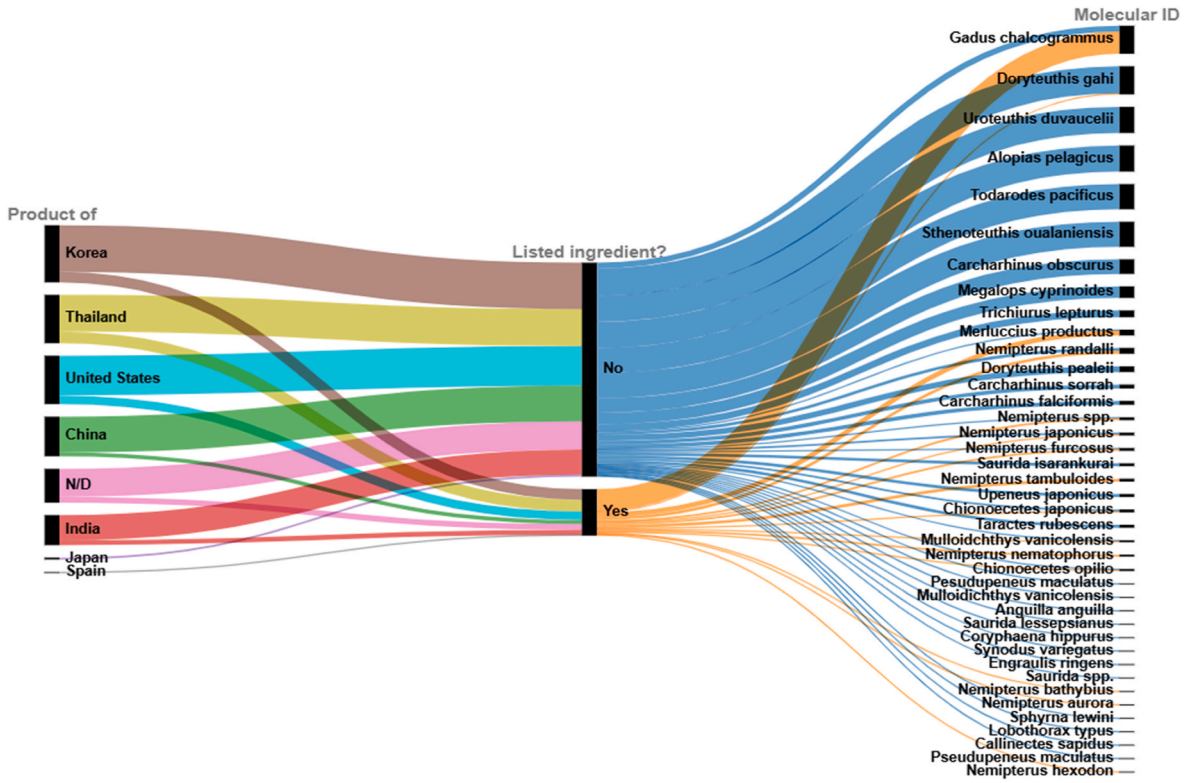

Fig. 2. Surimi DNA metabarcoding samples by 'Product of' country, if ingredient was listed on product packaging, and the molecular identification of the contained ingredient. N/D means no country listed on packaging.

(USDA 7 CFR Part 60), however, processed foods including surimi are exempt. The surimi industry is reliant on an increasing number of species from many countries (Leadbitter et al. 2020), adding pressure to fishery species that may already be overexploited or vulnerable to Illegal, Unreported, and Unregulated (IUU) fishing.

The US seeks to help address IUU fishing via the Seafood Import Monitoring Program (SIMP) which mandates formative seafood traceability requirements (NOAA 15 CFR part 902, Vol 81). The SIMP is a risk-based traceability program covering more than 1100 unique species that represent roughly half of US imports. The US is one of the world's largest importers of cephalopods (FAO 2025), yet cephalopods are not yet included in the SIMP. Expanding a focus to include squid may be beneficial as *I. argentinus* was identified as 29 % of frozen single species samples and *S. oualaniensis* detected in 60 % of surimi samples, two species caught in unregulated areas, often with a high risk of illegal fishing (Gleadall et al. 2024; Seto et al. 2023).

Conclusion

To our knowledge, this is the first study to analyze squid using DNA barcoding and surimi using DNA metabarcoding in the US marketplace. We found no instances of mislabeling of frozen squid products under current FDA guidance, with these products sold under a wide range of brands and grocers, and as the 'Product of' 12 countries and identified as 10 unique species. In contrast, only 72 % of surimi samples were detected to contain at least one species included on the ingredient list, but 95 % of samples were detected to contain undeclared species. Our findings indicate broad compliance with labeling guidance for US sold frozen squid products yet highlight the need for updated policy framework for blended seafood like surimi. Fortunately, such updating can be informed by the recent US National Aquatic eDNA Strategy (Goodwin et al., 2024), which provides a framework for leveraging DNA metabarcoding methods to enhance species-level verification processed seafood, a resource to help address gaps in FDA CPG 540.700 by enabling accurate identification of blended seafood products. Furthermore, our results reinforce our ongoing advocacy for widespread DNA-based seafood testing in Los Angeles (Willette et al., 2017, 2025) and more broadly, in the US seafood supply chain to improve labeling credibility, consumer confidence, and supply chain integrity.

CRediT authorship contribution statement

Demian A. Willette: Writing – review & editing, Writing – original draft, Visualization, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Margarita L. Joaquin:** Writing – review & editing, Project administration, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Partial funding for this project was provided from the Walton Family Foundation to Demian Willette and Species Tracks LLC. The study's results and outcomes were not shaped or influenced by this funder and this study was conducted in the high, rigorous, and consistent standard the same as all other research by Dr. Demian Willette.

Acknowledgements

We thank Leonardo Gonzalez-Smith, Sabriya Seid, Noopur Barve, Amina Jackson, Kate Eisen, and the Eisen Lab for assistance in the lab and/or constructive feedback on the manuscript. We also thank two anonymous reviewers and one editor for constructive feedback that improved the manuscript. We acknowledge research support from LMU Office of the Provost and LMU Seaver College of Science & Engineering. This work was partially funded by a grant from the Walton Family Foundation.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodcont.2025.111733.

Data availability

Data will be made available on request.

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