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DNA barcoding detects market substitution in North American seafood

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ABSTRACT

Seafood authentication and food safety concerns are a growing issue in today's global marketplace, although traditional morphology-based identification keys and existing molecular approaches have limitations for species identification. Recently, DNA barcoding has gained support as a rapid, cost-effective and broadly applicable molecular diagnostic technique for this purpose. However, the maturity of the barcode database as a tool for seafood authentication has yet to be tested using real market samples. The present case study was undertaken for this reason. Though the database is undergoing continual development, it was able to provide species matches of >97% sequence similarity for 90 of 91 samples tested. Twenty-five percent of the samples were potentially mislabeled, demonstrating that DNA barcodes are already a powerful tool for the identification of seafood to the species level. We conclude that barcodes have broad applicability for authenticity testing and the phylogeographic patterning of genetic diversity can also inform aspects of traceability.

1. Introduction

The increased public awareness of nutritional and environmental issues has resulted in a shift in consumer attitude towards seafood products. With importation and consumer consumption of seafood increasing, a growing number of fish species are being encountered in the market as a result of increased demand and the globalization of the seafood industry. Subsequent economic deception and food safety concerns are pushing the need for accurately labeled food products and full disclosure of product composition. A dramatic increase in media coverage involving cases of market substitution demonstrates that high quality, nutritious and "eco-friendly" food items are now a focal point for the educated consumer. In this regard, the authenticity and certification of fish products is particularly important when fresh or frozen cuts of fish are encountered because misrepresentation of the actual product, whether through intentional or non-intentional mislabeling, is known to occur (Marko et al., 2004). Unfortunately, consumers are unable to detect these cases given that recognizable external morphological features are typically removed when the fish is filleted or otherwise processed. The lack of morphological features that are traditionally used to identify animal species is a common problem with food products, making authenticity tests impossible without alternative identification methods.

Molecular diagnostic techniques have proven to be effective species identification tools, capable of bypassing the inherent problems of morphology-based identification methods. However, early macromolecular techniques, such as electrophoretic and immunological identification (Rehbein, 1990; Swart & Wilks, 1982), exhibited limitations of their own. For example, proteins of interest often denature during heating and/or processing, are tissue-specific, and are prone to contamination (Hofmann, 1987; Patterson & Jones, 1990), making these methods challenging to interpret and difficult to replicate. Today, DNA-based methods are more frequently employed for food authentication (Lockley & Bardsley, 2000). As with past electrophoretic and immunological methods, the use of DNA allows identification to proceed on samples lacking diagnostic morphological features.

The continually improving ability to analyze DNA has resulted in a large degree of success for DNA-based methods of authenticating animal meat products. Lockley and Bardsley (2000) summarize a growing library of authentication studies that utilize a variety of DNA-based methods to identify a wide range of meats, from fish and livestock, to a variety of game animals. The methods covered in these studies include DNA hybridization, species-specific polymerase chain reaction (PCR) primers, restriction fragment length polymorphism (RFLP) analysis, single strand conformational polymorphism (SSCP) analysis, random amplified polymorphic DNA (RAPD) analysis, and PCR product sequencing. While all of these methods hold both advantages and disadvantages (Table 1), an overarching problem lies in selecting an appropriate method from the multitude of potential analytical pathways available. Since the majority of methods are optimized for the identification of certain species, it is inappropriate to analyze a given sample with a method that was not designed for that species. Highly specific techniques therefore often require some prior knowledge of what the unknown sample may be in order to conduct the analysis

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Table 1
A comparison of DNA-based species identification techniques

	Applicable to degraded material	Low DNA requirement	Simple protocol	Mixture detection	Time efficient	No prior knowledge required	Reproducible between labs	Standardized across broad taxa
Hybridization	×			×				
Species-specific primer	×	×	×	×	×		×	
RFLP		×	×		×	×	×	
SSCP		×			×			
RAPD		×	×		×			
Traditional sequencing	×*	×	×		×	×	×	
DNA barcoding	×*	×	×		×	×	×	×

Techniques marked with an '×' indicate that they exhibit the corresponding feature.
* Only applies to small fragments in the case of severely degraded samples.

efficiently, and because these techniques are specialized for a specific group of animals, they do not necessarily address the breadth of species that may be encountered in today's global market place. Using these techniques on an unintended group poses a degree of risk for generating both false positive and false negative results. Until now there has been no global effort to provide a standardized approach to DNA-based authentication of animal food products.

Recently, DNA barcoding has gained considerable support as a rapid, cost-effective and broadly applicable tool for species identification. DNA barcoding targets a small standardized fragment of the cytochrome *c* oxidase I (COI) mitochondrial gene (Hebert, Cywinska, Ball, & deWaard, 2003; Hebert, Ratnasingham, & DeWaard, 2003) and as a molecular diagnostic technique, holds great promise (Dasmahapatra & Mallet, 2006). The target ~650 base pair fragment located at the 5′ end of the COI gene is PCR amplified and sequenced to produce reference sequences or "DNA barcodes" that act as molecular identification tags for each species profiled. DNA barcoding employs a standardized methodology to populate a publicly accessible database for species identification, one that is actively curated and explicitly derived from expert-identified reference materials. The scale of species coverage envisioned and the subsequent scope of potential applications to be supported distinguish DNA barcoding from previous molecular approaches. In 2005, fishes were selected as primary targets for global barcode coverage because of their socio-economic importance and more than 5000 species have currently been profiled. A growing body of literature on DNA barcoding demonstrates that the relatively short fragment of COI used for barcoding contains enough variation to accurately identify a large variety of animals to the species level (Waugh, 2007). This includes both freshwater (Hubert et al., 2008) and marine fishes (Rock et al., 2008; Spies, Gaichas, Stevenson, Orr, & Canino, 2006; Ward, Holmes, White, & Last, 2008; Ward, Zemlak, Innes, Last, & Hebert, 2005).

The concept of identifying unknown species with sequence data is not novel. "Forensically informative nucleotide sequencing" (FINS) was one of the earliest species diagnostic techniques for fish that utilized such an approach (Bartlett & Davidson, 1992). FINS involved the PCR amplification and sequencing of a mitochondrial cytochrome b gene fragment derived from the unknown sample, which was then compared to a database of reference sequences from known species in order to resolve the species identity of the unknown. Such sequencing approaches have been successful in identifying a variety of meats, using a variety of genetic markers (Bartlett & Davidson, 1991; Forrest & Carnegie, 1994; Matsunaga, Shibata, Yamada, Shinmura, & Chikuni, 1998; Unseld, Beyermann, Brandt, & Hiesel, 1995). While sequencing techniques are considered the most direct way to obtain a large amount of information, they were time consuming and expensive at the time when these studies were conducted. They also suffered (and continue to suffer) from a limited set of reference sequences for comparison. Nearly two decades after the initial development of FINS, improved technology has resulted in faster and more affordable sequencing capabilities. DNA barcoding now takes advantage of streamlined and inexpensive protocols (Ivanova, DeWaard, & Hebert, 2006; Ivanova, Zemlak, Hanner, & Hebert, 2007) that facilitate processing hundreds of thousands of samples per year within a single DNA barcoding core facility (Hajibabaei et al., 2005). Despite this trend toward automation and high-throughput, DNA barcoding remains very accessible for taxonomic, regulatory, or private purposes because specialized equipment, beyond that found in most modern molecular biology laboratories, is not required.

A fundamental aspect of DNA barcoding is that it seeks to extend the evidentiary value of each reference barcode sequence by incorporating a level of supplementary information not normally seen with sequence data. The addition of supplementary information is emphasized by the barcode of life data systems (BOLD; Ratnasingham & Hebert, 2007), a database that currently houses over 400,000 barcode sequences, representing approximately 40,000 species (as of May 2008). BOLD is structured to provide reference barcode records with a link to a voucher specimen housed in a public collection that has a taxonomic identification provided by an expert. BOLD also includes supplemental information involving collection event details (date, location, etc.), primer information, and the raw electropherogram trace files used to derive the assembled sequence profile. The transparency and traceability instilled into a reference barcode by the integration of this information opens it to be scrutinized and reviewed. Repeatability can be established as multiple laboratories will have the information necessary to independently process or re-analyze a given sample.

Typically, for previous molecular identification techniques, unknown sequences were queried against GenBank (Benson, Karsch-Mizrachi, Lipman, Ostell, & Wheeler, 2007) using the basic local alignment search tool (BLAST) algorithm (Altschul et al., 1997). Not surprisingly, accurate species identification hinged on the known records within GenBank having correct taxonomic designations and being error-free. Unfortunately, given the present day torrent of data generation, erroneous records have been known to make their way into public archives (Bridges, Roberts, Spooner, & Panchal, 2003; Forster, 2003; Harris, 2003; Nilsson et al., 2006; Ross & Murugan, 2006; Yao, Bravi, & Bandelt, 2004). Moreover, it is not usually possible to verify a suspect record, as the means to re-examine the raw sequence data or voucher specimen are not readily accessible from these archives.

The inability to verify the taxonomic identity of publicly archived sequences prompted a call for examined materials to be retained and accompanied by a "taxonomic affidavit" (Por, 2007). DNA barcoding's development of a higher data standard is in accordance with this plea, as orchestrated by the Consortium for the

Barcode of Life (CBOL), an international collaboration dedicated to the overall development of DNA barcoding. CBOL's effort to establish a higher data standard for reference DNA barcode sequences represents a paradigm shift in sequence archive philosophy towards emphasizing a more application driven use of sequence data. Because a questioned record can be verified and revised if necessary, the inclusion of a voucher specimen to underpin all reference DNA barcode records is a significant resource in practice.

Species identification using DNA barcodes relies on the observation that barcode sequence divergence within species is typically much lower than the divergence exhibited between species. Capitalizing on this observation, the barcode identification engine built into BOLD uses a genetic distance approach to compare and match unknown sequences to entries in the reference database. The barcode identification engine of BOLD is publicly accessible and allows users to query unknown sequences against either the full database or a reference subset of records that meet specific criteria outlined by Ratnasingham and Hebert (2007). As a workbench for the assembly of barcode profiles, BOLD promotes a community-based system of data curation that allows taxonomic experts to continually monitor the archive and make necessary corrections as new information becomes available. The "reference" partition of BOLD represents a vetted subset of the full database and requires that three or more conspecific specimens exhibiting less than 2% sequence divergence to be present before a given species can be included in the "reference" partition. Detected conflicts (different species exhibiting identical or nearly identical haplotypes) are excluded from the reference subset, while all available data is retained in the full database search option. Conflicts require careful validation to differentiate misidentification or laboratory errors from cases of valid haplotype sharing between two or more putative species. The latter occurrence is rare but is known to occur in a relatively few cases involving closely related species (e.g. Hubert et al., 2008; Spies et al., 2006). With the uptake of the barcode data standard by the taxonomic community and an iterative review mechanism in place, DNA barcodes hold a distinct advantage over other sequence databases with regards to data quality. This fact helps build confidence in barcode reference sequences, especially over time as the reference sequence library of the barcode database matures. Coordinated international efforts to compile barcode records for fish and seafood species, such as the fish barcode of life initiative (FISH-BOL, http://www.fishbol.org) and the marine barcode of life (MarBOL, http://www.marinebarcoding.org), will continue to strengthen the DNA barcode database, making it better suited for the demands of the global market.

The success of DNA barcoding thus far has caught the interest of agencies such as the US Food and Drug Administration (FDA) (Yancy et al., 2007). In a recent food poisoning investigation, DNA barcodes were used to help confirm the identity of toxic puffer fish in a Chicago market that had been illegally imported into the country mislabeled as "headless monkfish". The DNA barcodes were one piece of evidence in the joint investigation between the FDA and Chicago Department of Public Health that integrated evidence from multiple sources, including morphology and toxicology. Results from this investigation led to a recall of 282 cases of mislabeled product in three states and prompted the FDA to release public advisories about safe sources of puffer fish in the US (J. Deeds, personal communication, November 13, 2007). General interest in utilizing DNA barcoding as a tool in applied fields has been growing quickly (Dawnay, Ogden, McEwing, Carvalho, & Thorpe, 2007; Nelson, Wallman, & Dowton, 2007; Smith, McVeagh, & Steinke, 2008).

Here we develop a case study to evaluate the ability of DNA barcoding to identify the species of seafood products acquired directly from commercial markets and restaurants found in north eastern North America. A comparison of the BOLD and GenBank databases is made to evaluate their relative performance in generating positive matches for species identification.

2. Methods

Ninety-six samples of fish and seafood muscle tissue were acquired from commercial markets and restaurants in north eastern North America, from both Canada and the US. Upon collection, samples were stored in 95% ethanol at −20 °C until processed. Tissue of size 1–2 mm^3 was used for DNA extraction via extraction protocols detailed by Ivanova et al. (2006).

A 652 bp fragment from the 5′region of COI was PCR amplified using a forward and reverse primer cocktail (Table 2), C_FishF1t1 and C_FishR1t1 (Ivanova et al., 2007), appended with M13 tails to aid in sequencing (Messing, 1983). Each PCR reaction mixture consisted of 6.25 μl of 10% trehalose, 3.0 μl of ultrapure ddH_2O, 1.25 μl of 10× PCR buffer for Platinum® Taq (Invitrogen Inc.), 0.625 μl of 50 mM $MgCl_2$, 0.125 μl of each primer (10 μM), 0.0625 μl of 10 mM dNTP mix, 0.06 μl of Platinum® Taq DNA polymerase (Invitrogen Inc.), and 0.5–2.0 μl of template DNA. PCR amplification reactions were conducted on Eppendorf Mastercycler® gradient thermal cyclers (Brinkmann Instruments Inc.) The reaction program consisted of 2 min at 94 °C, followed by 35 cycles of 30 s at 94 °C, 40 s at 52 °C, and 1 min at 72 °C. Upon completion of the 35 cycles, the thermal program concluded with 10 min at 72 °C, followed by a hold at 4 °C.

PCR products were visualized on 2% agarose E-gel® 96 plates (Invitrogen Inc.). PCR products were labeled using the BigDye® Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems Inc.). Each cycle sequencing reaction mixture consisted of 5.0 μl of 10% trehalose, 0.917 μl of ultrapure ddH_2O, 1.917 μl of 5× buffer (400 mM Tris–HCl pH 9.0 and 10 mM $MgCl_2$), 1.0 μl of primer (10 μM; M13F or M13R), 0.167 μl of BigDye® (Applied Biosystems Inc.), and 1.5 μl of PCR product. Bi-directional sequencing reactions were carried out with the M13 primers (Table 2) and resolved using an ABI3730 capillary sequencer.

Bi-directional contig assembly was carried out using SeqScape v2.1.1 (Applied Biosystems Inc.). Identification of unknown samples was conducted using BLAST to search GenBank, and the BOLD

Table 2
PCR primer cocktail components and corresponding sequences

Primer		Sequence	
Cocktail name	Component name		
C_FishF1t1 (1:1 ratio)	VF2_t1	5'TGTAAAACGACGGCCAGTCAACCAACCACAAAGACATTGGCAC3'	(Ward et al. (2005))
	FishF2_t1	5'TGTAAAACGACGGCCAGTCGACTAATCATAAAGATATCGGCAC3'	(Ward et al. (2005))
C_FishR1t1 (1:1 ratio)	FishR2_t1	5'CAGGAAACAGCTATGACACTTCAGGGTGACCGAAGAATCAGAA3'	(Ward et al. (2005))
	FR1d_t1	5'CAGGAAACAGCTATGACACCTCAGGGTGTCCGAARAAYCARAA3'	(Ivanova et al., 2007)
M13F		5'TGTAAAACGACGGCCAGT3'	(Messing, 1983)
M13R		5'CAGGAAACAGCTATGAC3'	(Messing, 1983)

M13 tails are highlighted.

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Table 3
List of all samples in this case study that are suspected of being mislabeled

Sample number	Sold as	Identified as (BOLD)	Note
EMRKT006-07	Dockside classic sole	*Limanda aspera* Yellowfin sole	*L. aspera* is not listed in the FDA seafood list for ‘‘sole”. This species does appear on the CFIA list of acceptable names. However, this sample was collected in the United States.
EMRKT008-07	Red snapper, US wild caught	*Pristipomoides sieboldii* Lavender jobfish *Lutjanus aratus* Mullet snapper	*Lutjanus campechanus* is the accepted species for red snapper in the U.S.
EMRKT014-07	Sea bass sushi	*Morone chrysops* White bass	‘‘Sea bass” is not an acceptable name for *M. chrysops* according to both the FDA and CFIA seafood lists.
EMRKT016-07	Tobako flying fish roe	*Mallotus villosus* Capelin	Capelin is smelt, not flying fish.
EMRKT021-07	Red mullet, cooked	*Pseudupeneus maculates* Spotted goatfish	‘‘Red mullet” is not an acceptable name for *P. maculates* according to the FDA seafood list. This fish was sold as a Mediterranean fish, which *P. maculates* is not.
EMRKT022-07	Sea bass, cooked	*Morone saxatilis* Striped bass	‘‘Sea bass” is not an acceptable name for *M. saxatilis* according to both the FDA and CFIA seafood listss.
EMRKT025-07	Tai snapper sushi	*Pagrus major* Red seabream	‘‘Tai snapper” does not appear on either the FDA or CFIA seafood lists. However, ‘‘Tai” is listed as a vernacular name for *P. major* on FishBase.
EMRKT027-07	Red snapper, US wild caught	*Pristipomoides sieboldii* Lavender jobfish	*Lutjanus campechanus* is the accepted species for red snapper in the US.
EMRKT031-07	Basa fish filet	*Pangasius hypophthalmus* Swai/Sutchi catfish	*P. hypophthalmus* is not listed in the FDA seafood list for ‘‘basa”. This species does appear on the CFIA list of acceptable names. However, this sample was collected in the United States.
EMRKT032-07	Red snapper filet	*Sebastes fasciatus* Labrador redfish/Acadian redfish	*Lutjanus campechanus* is the accepted species for red snapper in the US.
EMRKT038-07	Red snapper	*Pinjalo lewisi* Slender pinjalo	*Lutjanus campechanus* is the accepted species for red snapper in the US.
EMRKT040-07	Boneless baccalo	*Theragra chalcogramma* Alaska Pollock	Strictly speaking, baccalo/bacalao is a common name for cod. However the term seems flexible given that bacalao has been explicitly specified as other fish (See Table 4 sample number EMRKT041-07)
EMRKT044-07	Halibut, alaska	*Hippoglossus hippoglossus* Atlantic halibut	Pacific halibut should be *Hippoglossus stenolepis*.
EMRKT046-07	Italian mackerel	*Dicentrarchus labrax* European Sea Bass	‘‘Italian mackerel” is not an acceptable name for *D. labrax* according to both the FDA and CFIA seafood lists. Mackerel and sea bass are different families.
EMRKT048-07	Kingfish	*Scomberomorus cavalla* King mackerel	*S. cavalla* is not listed in the FDA seafood list for ‘‘Kingfish”. This species does appear on the CFIA list of acceptable names. However, this sample was collected in the United States.
EMRKT050-07	White snapper	*Urophycis tenuis* White hake	‘‘White snapper” is not an acceptable name for *U. tenuis* according to both the FDA and CFIA seafood lists. Hake and snappers are different families.
EMRKT051-07	Red snapper	*Sebastes fasciatus* Labrador redfish/Acadian redfish	*Lutjanus campechanus* is the accepted species for red snapper in the US.
EMRKT053-07	White tuna sushi	*Oreochromis mossambicus* Mozambique tilapia	White tuna refers to albacore tuna (*Thunnus alalunga*)
EMRKT055-07	Red snapper sushi	*Gadus morhua* Atlantic cod	*Lutjanus campechanus* is the accepted species for red snapper in the US.
EMRKT064-07	Red snapper filet	*Lates niloticus* Lake Victoria perch/Nile perch	*Lutjanus campechanus* is the accepted species for red snapper in the US.
EMRKT066-07	California roll crab	*Theragra chalcogramma* Alaska pollock	This is not unusual. Pollock is often used in imitation crab and other imitation seafoods.
EMRKT082-07	Sea bass Chile	*Dissostichus mawsoni* Antarctic toothfish	Chilean sea bass refers to *Dissostichus eleginoides*.
EHKWX005-07	Halibut family	*Merluccius* paradoxus Deep-water Cape hake	*M. paradoxus* belongs to the family Merlucciidae, which are not considered halibut

The specific reasoning for the inclusion of each sample is noted.

identification engine to search barcode records within BOLD. Top species matches (highest percentage) obtained from both BLAST and BOLD for each specimen were compared to the relevant species name(s) corresponding to the recorded market name as derived from the FDA Center for Food Safety and Applied Nutrition Seafood List (http://www.cfsan.fda.gov/~frf/seaintro.html) and the Canadian Food Inspection Agency's (CFIA) List of Canadian Acceptable Common Names for Fish and Seafood (http://active.inspection.gc.ca/scripts/fispoi/fplist/fplist.asp?lang=e). For the purely organizational purposes of this study, we used a general rule that defined a top match with sequence similarity of at least 97% to indicate a potential species identity. Divergence thresholds for species identification were introduced in previous studies (Hebert, Stoeckle, Zemlak, & Francis, 2004; Lefebure, Douady, Gouy, & Gibiert, 2006), however, the 3% used here can be considered a relatively loose criterion. BOLD and GenBank rely on FishBase (http://www.fishbase.org) as a taxonomic authority for valid fish species names (Froese & Pauly, 2008). Species names in the FDA and CFIA tables that were inconsistent with currently accepted scientific names listed in FishBase were cross-referenced to an accepted species name based on known synonymies listed in FishBase. The identification of potentially mislabeled samples was based on a rigid and literal interpretation of the FDA and CFIA tables. Therefore some cases of potentially mislabeled fish are less egregious than others, and could even be considered acceptable under common consumer knowledge and expectations. This literal approach was used in order to ensure that the determination of authenticity was conducted consistently for all samples, which was particularly important in cases where a single market name applied to multiple species, or multiple naming categories existed for a species (e.g. common, market, and vernacular names).

3. Results and discussion

Ninety-one of the 96 unknown markets samples amplified successfully and were subsequently sequenced bi-directionally to assemble a full length COI barcode. When performing a BLAST search of GenBank, 24 sequences, representing an estimated 16 species, returned matches of less than 97% (range 82–96%) maximum identity. Of these same 24 samples, all but one of them returned a closer match with a different species when the BOLD identification engine was employed (sequence similarity 99–100%). The one exception was a sea urchin sushi sample which was matched to different sea urchin species via BLAST and BOLD at 90% and 88.71% similarity respectively, indicating that neither repository was sufficiently parameterized to enable a species level match (Ekrem, Willassen, & Stur, 2007). In all other cases, BOLD yielded greater resolution than GenBank. One specific discrepancy between GenBank and BOLD is illustrative: sample EMRKT082-07 was sold in the market as "Sea Bass Chile", which is a corresponding market name for "Patagonian toothfish" (*Dissostichus eleginoides*). BLAST suggests that this sample is correctly labeled as *D. eleginoides* (96% maximum identity). However, it is actually incorrect according to the BOLD identification engine, which identifies the sample as a different species with a 100% sequence match, the "Antarctic toothfish" (*Dissotichus mawsoni*).

GenBank and BOLD records are not mutually exclusive as there are some shared COI sequences between the two. However, a search of GenBank (as of May 2008) for COI sequences using all possible search terms (e.g. COI, cox1, and cytochrome *c* oxidase subunit 1) resulted in a hit of approximately 172,000 "core nucleotide" records, whereas BOLD as of the same date contained approximately 400,000 barcode sequences. While barcode sequences are eventually published to GenBank, the number differential reflects the fact that many BOLD records are part of ongoing research projects being conducted by the taxonomic community in support of DNA barcoding. Nonetheless, they are still utilized by the identification engine in a manner that is sensitive to the intellectual property rights of the researchers that generate them (i.e. sequence records remain private until they are published).

Twenty-three of the 91 sequenced samples are suspected to be mislabeled in some way (Table 3). Unless otherwise noted, acceptable matches were based on the name the samples were sold as appearing on one of the following two resources: the FDA seafood list (US) and the CFIA list of acceptable common names (Canada). Three samples suspected of being mislabeled represent differences between the US and Canada lists (EMRKT006-07, EMRKT031-07, and EMRKT048-07). For example, the market name "basa" includes *Pangasius hypophthalmus* in Canada, but not in the US. Therefore, a market sample labeled as "basa" from the US and identified as *P. hypophthalmus* (EMRKT031-07) via DNA barcoding is considered mislabeled.

The most commonly mislabeled fish in this study was "red snapper". Of the nine market samples sold as "red snapper", all from New York City, seven were not identified as *Lutjanus campechanus* (the accepted species name for "red snapper" sold in the US). This finding supports a previous study (Marko et al., 2004), which estimated that three quarters of all "red snappers" being sold in the US are mislabeled. However, there does not appear to be a single species that is consistently substituted for "red snapper" in these cases of mislabeling. The seven mislabeled "red snappers" were identified as belonging to five different species, each from a different genus.

Whether these samples were intentionally or unintentionally mislabeled, there exists a drastic economic impact. For example, two samples labeled as "red snapper" (EMRKT032-07 and EMRKT051-07) were identified as "Acadian redfish" (*Sebastes fasciatus*), and in 2006, US fisheries valued "red snapper" at \$2.93/lb while the generalized group "redfish", containing all Atlantic Ocean perches, was \$0.72/lb (Van Voorhees & Pritchard, 2007). It is not uncommon for a species of higher value to be substituted out for one of lower value (Hsieh, Chai, & Hwang, 2007). Similarly, DNA barcoding revealed that the "white tuna" sushi sample (EMRKT053-07), typically considered a more valuable sushi made from "albacore tuna", was instead "tilapia", a much less expensive fish.

Mislabeling on a subtle scale was also detected. One sample, EMRKT044-07, touted as "Alaskan halibut" (i.e. Pacific halibut, *Hippoglossus stenolepis*), was 100% identical to "Atlantic halibut" sequences (*Hippoglossus hippoglossus*). Though these two sister species are closely related, they do not share COI sequence haplotypes and are therefore easily discriminated by their barcodes. The BOLD identification engine was able to cleanly delineate several other samples of Pacific and Atlantic "halibut" into their respective species. The "Pacific halibut" is harvested with effective stock management practices, and is considered the "eco-friendly" substitute for the "Atlantic halibut", whose stock has collapsed and is now considered endangered (Brownstein, Lee, & Safina, 2003; Hilborn, Walters, & Ludwig, 1995). However, here we find exploitation of the endangered "Atlantic halibut" in place of the "eco-friendly" Pacific species, possibly hidden behind mislabeling.

In other cases, mislabeled samples appeared correct at first due to the use of common, market, or vernacular names that can be applied to multiple species simultaneously under current regulatory frameworks. These cases can be convoluted and typically differ from one country to the next. One sample labeled as "red mullet" (EMRKT021-07) was identified as "spotted goatfish" (*Pseudupeneus maculatus*) by its barcode. The difficulty is that "red mullet" is used as a vernacular name for "red goatfish" (*Mullus auratus*), and for several other goatfish in general. However, a specific search for *P.*

Table 4
List of all identification results using the BOLD identification engine (both reference subset and full database conditions) and a BLAST search of GenBank

Sample number	Sold as	Species identification		
		BOLD reference subset	BOLD full database	GenBank (BLAST)
EMRKT001-07	red snapper filet	*Lutjanus argentimaculatus* (89.35%) Mangrove red snapper	*Lutjanus campechanus* (100%) Red snapper/Northern red snapper *Lutjanus vivanus* (100%) Silk snapper	Lutjanus argentimaculatus (89%) Mangrove red snapper
EMRKT002-07	Tilapia filet, farm-raised China	*Oreochromis sp.* (93.21%)	*Oreochromis niloticus* (100%) Nile tilapia	*Oreochromis niloticus* (100%) Nile tilapia
EMRKT003-07	Grey sole filet	No sequence		
EMRKT004-07	Scrod filet	*Gadus morhua* (100%) Atlantic cod	*Gadus morhua* (100%) Atlantic cod	*Gadus morhua* (100%) Atlantic cod
EMRKT005-07	Flounder filet	*Parophrys vetulus* (94.14%) English sole	*Pseudopleuronectes americanus* (100%) Blackback flounder/Winter flounder	*Platichthys bicoloratus* (92%) Stone flounder
EMRKT006-07	Dockside classic sole	*Limanda limanda* (98.07%) Common dab	*Limanda aspera* (100%) Yellowfin sole	*Platichthys bicoloratus* (90%) Stone flounder
EMRKT007-07	Cod filet, US wild caught	*Gadus morhua* (100%) Atlantic cod	*Gadus morhua* (100%) Atlantic cod	*Gadus morhua* (99%) Atlantic cod
EMRKT008-07	Red snapper, US wild caught	*Pristipomoides auricilla* (97.6%) Goldflag jobfish	*Pristipomoides sieboldii* (100%) Lavender jobfish *Lutjanus aratus* (100%) Mullet snapper	*Pristipomoides sieboldii* (99%) Lavender jobfish
EMRKT009-07	Turbot, Greenland	*Reinhardtius hippoglossoides* (100%) Greenland turbot/Greenland halibut	*Reinhardtius hippoglossoides* (100%) Greenland turbot/Greenland halibut	*Reinhardtius hippoglossoides* (100%) Greenland turbot/Greenland halibut
EMRKT010-07	Monkfish, US	*Lophius americanus* (100%) Monkfish/American angler	*Lophius americanus* (100%) Monkfish/American angler	*Lophius americanus* (99%) Monkfish/American angler
EMRKT011-07	Grey sole filet	No sequence		
EMRKT012-07	Flounder	*Parophrys vetulus* (94.14%) English sole	*Pseudopleuronectes americanus* (100%) Blackback flounder/Winter flounder	*Platichthys bicoloratus* (92%) Stone flounder
EMRKT013-07	Yellowtail sushi	*Seriola hippos* (92.74%) Samson fish	*Seriola lalandi* (99.85%) Yellowtail amberjack *Mugil cephalus* (99.85%) Striped Mullet	*Seriola lalandi* (94%) Yellowtail amberjack
EMRKT014-07	Sea bass sushi	*Morone chrysops* (99.37%) White bass	*Morone chrysops* (99.37%) White bass	*Morone saxatilis* (99%) Striped bass
EMRKT015-07	Mackerel sushi	*Scomber scombrus* (100%) Atlantic mackerel	*Scomber scombrus* (100%) Atlantic mackerel	*Scomber scombrus* (99%) Atlantic mackerel
EMRKT016-07	Tobako flying fish roe	*Mallotus villosus* (97.5%) Capelin	*Mallotus villosus* (97.5%) Capelin	*Osmerus mordax* (87%) Rainbow smelt
EMRKT017-07	Pickled herring	No sequence		
EMRKT018-07	Monkfish	*Lophius americanus* (100%) Monkfish/American angler	*Lophius americanus* (100%) Monkfish/American angler	*Lophius americanus* (99%) Monkfish/American angler
EMRKT019-07	Yellowtail tuna	*Thunnus obesus* (100%) Bigeye tuna	*Thunnus albacares* (100%) Yellowfin tuna *Thunnus obesus* (100%) Bigeye tuna	Thunnus albacares (100%) Yellowfin tuna
EMRKT020-07	Frozen chilean sea bass	*Dissostichus mawsoni* (96.6%) Antarctic toothfish	*Dissostichus eleginoides* (99%) Patagonian toothfish	*Dissostichus eleginoides* (99%) Patagonian toothfish
EMRKT021-07	Red mullet, cooked	*Pseudupeneus maculates* (99.85%) Spotted goatfish	*Pseudupeneus maculates* (99.85%) Spotted goatfish	*Parupeneus indicus* (85%) Indian goatfish
EMRKT022-07	Sea bass, cooked	*Morone saxatilis* (99.37%) Striped bass	*Morone saxatilis* (99.37%) Striped bass	*Morone saxatilis* (89%) Striped bass
EMRKT023-07	Mackerel sushi	*Scomber scombrus* (99.85%) Atlantic mackerel	*Scomber scombrus* (99.85%) Atlantic mackerel	*Scomber scombrus* (99%) Atlantic mackerel
EMRKT024-07	Fluke sushi	Paralichthys dentatus (99.85%) Summer flounder	Paralichthys dentatus (99.85%) Summer flounder	*Paralichthys olivaceus* (87%) Olive flounder/Bastard halibut
EMRKT025-07	Tai snapper sushi	*Pagrus major* (99.63%) Red seabream	*Pagrus major* (99.63%) Red seabream	*Pagrus major* (97%) Red seabream
EMRKT026-07	Turbot filet, Canada	*Reinhardtius hippoglossoides* (100%) Greenland turbot/Greenland halibut	*Reinhardtius hippoglossoides* (100%) Greenland turbot/Greenland halibut	*Reinhardtius hippoglossoides* (99%) Greenland turbot/Greenland halibut
EMRKT027-07	Red snapper, US wild caught	*Pristipomoides auricilla* (97.76%) Goldflag jobfish	*Pristipomoides sieboldii* (100%) Lavender jobfish *Lutjanus aratus* (100%) Mullet snapper	*Pristipomoides sieboldii* (100%) Lavender jobfish
EMRKT028-07	Flounder filet	*Parophrys vetulus* (95.5%) English sole	*Lepidopsetta bilineata* (99.37%) Rock sole	*Platichthys bicoloratus* (92%) Stone flounder
EMRKT029-07	Salmon sushi	*Salmo salar* (99.53%) Atlantic salmon	*Salmo salar* (99.53%) Atlantic salmon	*Salmo salar* (99%) Atlantic salmon
EMRKT030-07	Whiting, whole fish	*Merluccius productus* (99.22%) Pacific whiting/Northern Pacific hake	*Merluccius productus* (99.22%) Pacific whiting/Northern Pacific hake	*Merluccius gayi* (98%) Chilean Hake/South Pacific hake
EMRKT031-07	Basa fish filet	*Pangasius hypophthalmus* (99.85%) Swai/Sutchi catfish	*Pangasius hypophthalmus* (99.85%) Swai/Sutchi catfish	*Pangasius hypophthalmus* (99%) Swai/Sutchi catfish
EMRKT032-07	Red snapper filet	*Sebastes fasciatus* (100%) Labrador redfish/Acadian redfish	*Sebastes fasciatus* (100%) Labrador redfish/Acadian redfish	*Sebastes mentella* (100%) Deepwater redfish *Sebastes norvegicus* (100%) Golden redfish *Sebastes fasciatus* (100%) Labrador redfish/Acadian redfish
EMRKT033-07	Swordfish	*Xiphias gladius* (100%) Swordfish	*Xiphias gladius* (100%) Swordfish	*Xiphias gladius* (100%) Swordfish

EMRKT034-07	Flounder	*Hippoglossoides elassodon* (100%) Flathead sole	*Hippoglossoides elassodon* (100%) Flathead sole	*Platichthys bicoloratus* (90%) Stone flounder
EMRKT035-07	Scrod, US wild-caught	*Gadus morhua* (98.48%) Atlantic cod	*Theragra chalcogramma* (99.69%) Alaska pollock	*Theragra finnmarchica* (99%) Norwegian pollock *Theragra chalcogramma* (99%) Alaska pollock
EMRKT036-07	Catfish	*Ictalurus punctatus* (99.37%) Channel catfish	*Ictalurus punctatus* (99.37%) Channel catfish	*Ictalurus punctatus* (99%) Channel catfish
EMRKT037-07	Smoked herring	No sequence		
EMRKT038-07	Red snapper	*Lutjanus erythropterus* (92.44%) Crimson snapper	*Pinjalo lewisi* (100%) Slender pinjalo	*Lutjanus adetii* (90%) Yellow-banded snapper
EMRKT039-07	Yellowfin tuna	*Thunnus obesus* (100%) Bigeye tuna	*Thunnus albacares* (100%) Yellowfin tuna *Thunnus obesus* (100%) Bigeye tuna	*Thunnus albacares* (100%) Yellowfin tuna
EMRKT040-07	Boneless baccalo	*Gadus morhua* (98.65%) Atlantic cod	*Theragra chalcogramma* (100%) Alaska pollock	*Theragra finnmarchica* (100%) Norwegian pollock *Theragra chalcogramma* (100%) Alaska pollock
EMRKT041-07	Bacalao pollock filets	*Gadus morhua* (97.81%) Atlantic cod	*Theragra chalcogramma* (99.23%) Alaska pollock	*Theragra finnmarchica* (99%) Norwegian pollock *Theragra chalcogramma* (99%) Alaska pollock
EMRKT042-07	Madai sushi	*Pagrus major* (100%) Red seabream	*Pagrus major* (100%) Red seabream	*Pagrus major* (100%) Red seabream
EMRKT043-07	Chilean sea bass, grilled	*Dissostichus mawsoni* (96.45%) Antarctic toothfish	*Dissostichus eleginoides* (100%) Patagonian toothfish	*Dissostichus eleginoides* (100%) Patagonian toothfish
EMRKT044-07	Halibut, alaska	*Hippoglossus hippoglossus* (100%) Atlantic halibut	*Hippoglossus hippoglossus* (100%) Atlantic halibut	*Hippoglossus hippoglossus* (100%) Atlantic halibut
EMRKT045-07	Mako shark	*Lamna ditropis* (86.57%) Salmon shark	*Isurus oxyrinchus* (99.85%) Shortfin mako shark	*Carcharodon carcharias* (86%) Great white shark
EMRKT046-07	Italian mackerel	*Morone saxatilis* (86.57%) Striped bass	*Dicentrarchus labrax* (97.46%) European Sea Bass	*Eutaeniophorus sp.* (82%) *Cetostoma regain* (82%) Pink flabby whalefish
EMRKT047-07	Tilefish	*Girella tricuspidata* (84.88%) Luderick	*Lopholatilus villarii* (98.77%) Tile fish	*Lopholatilus villarii* (98%) Tile fish
EMRKT048-07	Kingfish	Scomberomorus munroi (88.43%) Australian spotted mackerel	*Scomberomorus cavalla* (100%) King mackerel	*Scomberomorus cavalla* (100%) King mackerel
EMRKT049-07	Skate	*Rajella bathyphila* (92.85%) Deepwater ray	*Leucoraja ocellata* (100%) Winter skate	*Rajella bigelowi* (91%) Bigelow s ray
EMRKT050-07	White snapper	*Urophycis chuss* (92.13%) Red hake	*Urophycis tenuis* (100%) White hake	*Urophycis cirrata* (91%) Gulf Hake
EMRKT051-07	Red snapper	*Sebastes fasciatus* (100%) Labrador redfish/Acadian redfish	*Sebastes fasciatus* (100%) Labrador redfish/Acadian redfish	*Sebastes mentella* (100%) Deepwater redfish *Sebastes norvegicus* (100%) Golden redfish *Sebastes fasciatus* (100%) Labrador redfish/Acadian redfish
EMRKT052-07	Salmon sushi	*Salmo salar* (99.38%) Atlantic salmon	*Salmo salar* (99.38%) Atlantic salmon	*Salmo salar* (99 %) Atlantic salmon
EMRKT053-07	White tuna sushi	*Oreochromis sp.* (93.52%)	*Oreochromis mossambicus* (100%) Mozambique tilapia	*Oreochromis mossambicus* (99%) Mozambique tilapia
EMRKT054-07	Tuna sushi	*Thunnus obesus* (100%) Bigeye tuna	*Thunnus albacares* (100%) Yellowfin tuna *Thunnus obesus* (100%) Bigeye tuna	*Thunnus albacares* (100%) Yellowfin tuna
EMRKT055-07	Red snapper sushi	*Gadus morhua* (100%) Atlantic cod	*Gadus morhua* (100%) Atlantic cod	*Gadus morhua* (100%) Atlantic cod
EMRKT056-07	Chilean sea bass, Chile	*Dissostichus mawsoni* (96.45%) Antarctic toothfish	*Dissostichus eleginoides* (100%) Patagonian toothfish	*Dissostichus eleginoides* (100%) Patagonian toothfish
EMRKT057-07	Atlantic cod, wild	*Gadus morhua* (100%) Atlantic cod	*Gadus morhua (*100%) Atlantic cod	*Gadus morhua (*100%) Atlantic cod
EMRKT058-07	Atlantic halibut	*Hippoglossus hippoglossus* (100%) Atlantic halibut	*Hippoglossus hippoglossus* (100%) Atlantic halibut	*Hippoglossus hippoglossus* (100%) Atlantic halibut
EMRKT059-07	Red snapper, whole fish, Panama	*Lutjanus argentimaculatus* (89.35%) Mangrove red snapper	*Lutjanus campechanus* (100%) Northern red snapper *Lutjanus vivanus* (100%) Silk snapper	Lutjanus argentimaculatus (89%) Mangrove red snapper
EMRKT060-07	Black sea bass, whole fish	*Centropristis striata* (100%) Black sea bass	*Centropristis striata* (100%) Black sea bass	*Ptereleotris zebra* (83%) Zebra barred dartfish
EMRKT061-07	Ocean perch filet	*Sebastes fasciatus* (100%) Labrador redfish/Acadian redfish	*Sebastes fasciatus* (100%) Labrador redfish/Acadian redfish	*Sebastes mentella* (100%) Deepwater redfish *Sebastes norvegicus* (100%) Golden redfish *Sebastes fasciatus* (100%) Labrador redfish/Acadian redfish
EMRKT062-07	Chili sea bass	*Dissostichus mawsoni* (96.6%) Antarctic toothfish	*Dissostichus eleginoides* (100%) Patagonian toothfish	*Dissostichus eleginoides* (99%) Patagonian toothfish
EMRKT063-07	Cod filet	*Gadus macrocephalus* (100%) Pacific cod *Gadus ogac* (100%) Greenland cod	*Gadus macrocephalus* (100%) Pacific cod *Gadus ogac* (100%) Greenland cod Theragra chalcogramma (99.69%) Alaska pollock	*Gadus ogac* (100%) Greenland cod *Gadus macrocephalus* (100%) Pacific cod
EMRKT064-07	Red snapper filet	*Lates niloticus* (99.22%) Lake Victoria perch/Nile perch	*Lates niloticus* (99.22%) Lake Victoria perch/Nile perch	*Lates niloticus* (100%) Lake Victoria perch/Nile perch
EMRKT065-07	Sea urchin sushi	*Heliocidaris cf.erythrogramma* (84.75%)	*Hemicentrotus pulcherrimus* (88.71%)	*Strongylocentrotus pallidus* (90%)

EMRKT066-07	California roll crab	*Gadus morhua* (98.65%) Atlantic cod	*Theragra chalcogramma* (100%) Alaska pollock	*Theragra finnmarchica* (100%) Norwegian pollock *Theragra chalcogramma* (100%) Alaska pollock
EMRKT067-07	Salmon	*Salmo salar* (100%) Atlantic salmon	*Salmo salar* (100%) Atlantic salmon	*Salmo salar* (99%) Atlantic salmon
EMRKT068-07	Tilapia	*Oreochromis sp.* (100%)	*Oreochromis sp.* (100%)	*Oreochromis aureus* (99%) Blue tilapia
EMRKT069-07	Basa Fillet Vietnam	*Pangasius hypophthalmus* (100%) Swai/Sutchi catfish	*Pangasius hypophthalmus* (100%) Swai/Sutchi catfish	*Pangasius hypophthalmus* (99%) Swai/Sutchi catfish
EMRKT070-07	Basa Fillet New Zealand	*Pangasius hypophthalmus* (100%) Swai/Sutchi catfish	*Pangasius hypophthalmus* (100%) Swai/Sutchi catfish	*Pangasius hypophthalmus* (99%) Swai/Sutchi catfish
EMRKT071-07	Basa Fillet Vietnam	*Pangasius hypophthalmus* (100%) Swai/Sutchi catfish	*Pangasius hypophthalmus* (100%) Swai/Sutchi catfish	*Pangasius hypophthalmus* (99%) Swai/Sutchi catfish
EMRKT072-07	Basa Fillet New Zealand	*Pangasius hypophthalmus* (100%) Swai/Sutchi catfish	*Pangasius hypophthalmus* (100%) Swai/Sutchi catfish	*Pangasius hypophthalmus* (99%) Swai/Sutchi catfish
EMRKT073-07	Pollock	No sequence		
EMRKT074-07	Pollock	*Gadus morhua* (98.65%) Atlantic cod	*Theragra chalcogramma* (100%) Alaska pollock	*Theragra finnmarchica* (100%) Norwegian pollock *Theragra chalcogramma* (100%) Alaska pollock
EMRKT075-07	Sole	*Limanda limanda* (98.07%) Common dab	*Limanda aspera* (100%) Yellowfin sole	*Platichthys bicoloratus* (90%) Stone flounder
EMRKT076-07	Pollock	*Gadus morhua* (98.65%) Atlantic cod	*Theragra chalcogramma* (100%) Alaska pollock	*Theragra finnmarchica* (99%) Norwegian pollock *Theragra chalcogramma* (99%) Alaska Pollock
EMRKT077-07	Sole	*Limanda limanda* (98.65%) Common dab	*Limanda aspera* (99.23%) Yellowfin sole	*Platichthys bicoloratus* (90%) Stone flounder
EMRKT078-07	Basa Fillet Vietnam	*Pangasius hypophthalmus* (100%) Swai/Sutchi catfish	*Pangasius hypophthalmus* (100%) Swai/Sutchi catfish	*Pangasius hypophthalmus* (99%) Swai/Sutchi catfish
EMRKT079-07	Basa Fillet Vietnam	*Pangasius hypophthalmus* (99.85%) Swai/Sutchi catfish	*Pangasius hypophthalmus* (99.85%) Swai/Sutchi catfish	*Pangasius hypophthalmus* (99%) Swai/Sutchi catfish
EMRKT080-07	Rainbow trout Canada	*Oncorhynchus clarki* (95.53%) Cutthroat trout	*Oncorhynchus mykiss* (100%) Rainbow trout	*Oncorhynchus mykiss* (100%) Rainbow trout
EMRKT081-07	Rainbow trout Canada	*Oncorhynchus clarki* (95.68%) Cutthroat trout	*Oncorhynchus mykiss* (100%) Rainbow trout	*Oncorhynchus mykiss* (100%) Rainbow trout
EMRKT082-07	Sea bass Chile	*Dissostichus mawsoni* (100%) Antarctic toothfish	*Dissostichus mawsoni* (100%) Antarctic toothfish	*Dissostichus eleginoides* (96%) Antarctic toothfish
EMRKT083-07	Basa Fillet Vietnam	*Pangasius hypophthalmus* (100%) Swai/Sutchi catfish	*Pangasius hypophthalmus* (100%) Swai/Sutchi catfish	*Pangasius hypophthalmus* (99%) Swai/Sutchi catfish
EMRKT084-07	Rainbow trout Canada	*Oncorhynchus clarki* (95.83%) Cutthroat trout	*Oncorhynchus mykiss* (100%) Rainbow trout	*Oncorhynchus mykiss* (100%) Rainbow trout
EMRKT085-07	Rainbow trout Canada	*Oncorhynchus clarki* (95.68%) Cutthroat trout	*Oncorhynchus mykiss* (100%) Rainbow trout	*Oncorhynchus mykiss* (100%) Rainbow trout
EMRKT086-07	Dory Vietnam	*Pangasius hypophthalmus* (100%) Swai/Sutchi catfish	*Pangasius hypophthalmus* (100%) Swai/Sutchi catfish	*Pangasius hypophthalmus* (99%) Swai/Sutchi catfish
EMRKT087-07	Dory Vietnam	*Pangasius hypophthalmus* (100%) Swai/Sutchi catfish	*Pangasius hypophthalmus* (100%) Swai/Sutchi catfish	*Pangasius hypophthalmus* (99%) Swai/Sutchi catfish
EMRKT088-07	Atlantic Salmon	*Salmo salar* (99.38%) Atlantic salmon	*Salmo salar* (99.38%) Atlantic salmon	*Salmo salar* (100%) Atlantic salmon
EMRKT089-07	White Bass	*Morone chrysops* (99.33%) White bass	*Morone chrysops* (99.33%) White bass	*Morone saxatilis* (99%) Striped bass
EMRKT090-07	White Bass	*Morone chrysops* (99.37%) White bass	*Morone chrysops* (99.37%) White bass	*Morone saxatilis* (99%) Striped bass
EMRKT091-07	Tilapia	*Oreochromis sp.* (100%)	*Oreochromis niloticus* (100%) Nile tilapia	*Oreochromis aureus* (99%) Blue tilapia
EMRKT092-07	Tilapia	*Oreochromis sp.* (93.21%)	*Oreochromis niloticus* (100%) Nile tilapia *Oreochromis aureus x Oreochromis niloticus* (100%) Blue/Nile tilapia hybrid	*Oreochromis niloticus* (100%) Nile tilapia *Oreochromis aureus x Oreochromis niloticus* (100%) Blue/Nile tilapia hybrid
EHKWX004-07	Sole	*Microstomus pacificus* (100%) Dover Sole	*Microstomus pacificus* (100%) Dover Sole	*Reinhardtius hippoglossoides* (89%) Greenland turbot/Greenland halibut
EHKWX005-07	Halibut family	*Merluccius paradoxus* (100%) Deep-water Cape hake	*Merluccius paradoxus* (100%) Deep-water Cape hake	*Merluccius merluccius* (93%) European hake
EHKWX007-07	Salmon whole frozen	*Oncorhynchus gorbuscha* (99.85%) Pink Salmon	*Oncorhynchus gorbuscha* (99.85%) Pink Salmon	*Oncorhynchus gorbuscha* (99%) Pink Salmon
EHKWX008-07	Haddock	*Melanogrammus aeglefinus* (100%) Haddock	*Melanogrammus aeglefinus* (100%) Haddock	*Melanogrammus aeglefinus* (99%) Haddock

Common/market names provided are based on the FDA seafood list if available, otherwise common names from FishBase (http://www.fishbase.org) are used. If names differ between the FDA seafood list and FishBase, both names are listed. For BOLD identifications, top sequence matches are shown with sequence similarity percentages displayed in parentheses. Similarly for the BLAST search of GenBank, top matches by maximum score are shown with maximum identity percentages displayed in parentheses. Sample numbers listed in bold print were collected in the US. Sample numbers listed in italicized print were collected in Canada. Shaded samples are the potentially mislabeled samples that appear in Table 3.

maculatus in the FDA seafood list revealed that "red mullet" is not an acceptable market name for this particular species in the US. Furthermore, this sample was sold as a fish from the Mediterranean, but *P. maculatus* is distributed through the western Atlantic, particularly the Caribbean.

As another example of convoluted market nomenclature, the sample labeled "kingfish" (EMRKT048-07) was identified by barcoding as *Scomberomorus cavalla* (accepted common name "king mackerel"). When "kingfish" is entered into the FDA seafood list, *S. cavalla* is not listed as a possibility, though this sample is also notable as one of the three differences between the FDA and CFIA lists in this case study. However by the FDA list, "kingfish" is included as a vernacular name for *Scomberomorus regalis*, which shares a common name ("king mackerel") and market name

("Spanish mackerel") with *S. cavalla*. In the US, the market name "kingfish" refers to four species from the genus *Menticirrhus*. The ambiguity and confusion that can result from the existing national market nomenclature in a global economy stresses the need for current regulatory lists to be reviewed and revised. Indeed, those tasked with monitoring the international wildlife trade are openly calling for the adoption of scientific names in commerce labeling (Gerson et al., 2008).

The five samples that failed to amplify included a pair of "grey sole" filets, a smoked "herring", a pickled "herring", and "pollock". It is unlikely that these failures were the result of incompatible PCR primers, as all tentative species encompassed by these samples have barcode sequences in BOLD and have been successfully amplified in previous projects included in the database. There may be some concern as to whether the DNA was degraded and unrecoverable due to processing in the case of the smoked fish. However, previous studies have had success with smoked fish (Smith et al., 2008). Degradation of samples caused by processing, or otherwise poor quality DNA extractions, may also be addressed by the application of mini-barcodes (Hajibabaei et al., 2006).

Identification results for all samples are detailed in Table 4. Two identifications are provided for BOLD, one based on the full database and the other based on the reference subset of the database. Since the reference library is still being constructed and is highly conservative, the full database still provides valuable information, particularly in cases where the reference database does not provide a close match (e.g. >97% similarity). Current cases where the BOLD identification engine reports multiple species with the same sequence similarity are records that are pending review and possible revision.

4. Conclusion

The ability of DNA barcoding to detect mislabeled seafood products in this case study revealed a number of implications. The "red snapper" and "white tuna" sushi examples both draw attention to the economic impact of substitution, with high market value seafood products being substituted by a species of lesser value. Mislabeled products can also hamper stock management efforts, as seen with the supposed "Pacific halibut" that turns out to be an endangered "Atlantic halibut". Beyond legal and conservation implications, DNA barcoding will help provide a clear picture of what species are being harvested and to some extent, from where. This information will provide a foundation of increased resolution from which to examine overall patterns of exploitation. This is a pressing concern across all fish species currently being harvested, as a global collapse of all fisheries is expected by the mid-21st century (Worm et al., 2006).

The ambiguity and redundancy in regulatory lists, highlighted by a number of the market samples, results in a system that is inundated by multiple paths of legal substitution, therefore making it difficult for consumers to have confidence in what they are paying for. As an example, there are 33 species in the FDA seafood list that can be sold under the market name "grouper", and some are more valuable than others. Consumers want to be confident that they are receiving what they pay for, and a move away from using colloquial names in favor of labeling with scientific names would offer numerous benefits (as noted by Gerson et al., 2008). Further complications were evident with samples such as the "basa" that was *acceptable* in Canada, but considered *mislabeled* in the US. Both of these issues stress the need for the review and harmonization of regulatory lists. DNA barcoding offers a new level of precision in the application of species names, which is increasingly important in the expanding international market. In addition, revisions to regulatory lists supported by DNA barcoding will provide the industry and regulatory agencies with a means of authenticity testing and options for product authenticity programs. In turn, this would complement the commitment towards high ethical standards seen in trade organization programs such as the National Fisheries Institute's Economic Integrity Initiative.

DNA barcodes are emerging as a powerful tool for all parties concerned with food authentication or food safety, as well as those concerned with other aspects of fisheries management (Costa & Carvalho, 2007). The ease of generating DNA barcodes and a focus on high quality data records instill increasing confidence in the technique. Having the option to review a barcode sequence via the linked voucher specimen or other accompanying information is a potent advantage. With more than 5000 of the world's estimated 30,000 species of fishes currently profiled in BOLD, the broad identification of commercially relevant fish taxa was possible with DNA barcodes, allowing BOLD to outperform GenBank in terms of the number of species that could be accurately identified. The utility of barcoding will continue to grow as species coverage in the database increases (Ekrem et al., 2007) and in time, with the adoption of barcoding as an authentication tool, perhaps it will be possible to discourage seafood substitution in the marketplace. While the data standard of DNA barcoding will ensure that higher quality COI sequences are deposited in GenBank as the relevant studies generating these sequences are published, the BOLD database will serve as the primary resource for identification purposes for the foreseeable future because the identification engine on BOLD provides prepublication query access to this accumulating body of barcode survey data. The obvious strengths of DNA barcoding continue to draw significant interest from the applied fields (Dawnay et al., 2007; Yancy et al., 2007) and the outlook for barcoding as a regulatory tool is positive, allowing future practices to better address issues of market cost, safety, and environmental impact.

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