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FISH-BOL and seafood identification: Geographically dispersed case studies reveal systemic market substitution across Canada

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Abstract

Background and aims. The Fish Barcode of Life campaign involves a broad international collaboration among scientists working to advance the identification of fishes using DNA barcodes. With over 25% of the world's known ichthyofauna currently profiled, forensic identification of seafood products is now feasible and is becoming routine.

Materials and methods. Driven by growing consumer interest in the food supply, investigative reporters from five different media establishments procured seafood samples ($n = 254$) from numerous retail establishments located among five Canadian metropolitan areas between 2008 and 2010. The specimens were sent to the Canadian Centre for DNA Barcoding for analysis. By integrating the results from these individual case studies in a summary analysis, we provide a broad perspective on seafood substitution across Canada.

Results. Barcodes were recovered from 93% of the samples ($n = 236$), and identified using the Barcode of Life Data Systems "species identification" engine (www.barcodinglife.org). A 99% sequence similarity threshold was employed as a conservative matching criterion for specimen identification to the species level. Comparing these results against the Canadian Food Inspection Agency's "Fish List" a guideline to interpreting "false, misleading or deceptive" names (as per s 27 of the Fish Inspection regulations) demonstrated that 41% of the samples were mislabeled. Most samples were readily identified; however, this was not true in all cases because some samples had no close match. Others were ambiguous due to limited barcode resolution (or imperfect taxonomy) observed within a few closely related species complexes. The latter cases did not significantly impact the results because even the partial resolution achieved was sufficient to demonstrate mislabeling.

Conclusion. This work highlights the functional utility of barcoding for the identification of diverse market samples. It also demonstrates how barcoding serves as a bridge linking scientific nomenclature with approved market names, potentially empowering regulatory bodies to enforce labeling standards. By synchronizing taxonomic effort with sequencing effort and database curation, barcoding provides a molecular identification resource of service to applied forensics.

Keywords: *DNA barcoding, cytochrome* c *oxidase subunit I, seafood mislabeling, market substitution, Barcode of Life Data Systems*

Introduction

The intentional mislabeling of seafood with a product of lesser value constitutes a growing form of economic adulteration that is of concern for fisheries resource management worldwide (Jacquet and Pauly 2008). Reasons for substitution include high demand with limited supply, high profit incentive, an increase in international trade of processed foods, and lack of regulation enforcement and implementation (Miller and Mariani 2010). Seafood products have been found mislabeled at high levels in North America and Europe. For example, DNA-based approaches have demonstrated that between 60 and 94% of fishes labeled as Red Snapper *Lutjanus campechanus* (Poey)

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for sale in the USA were mislabeled (Marko et al. 2004), as was 25% of various species obtained from markets and restaurants in New York City (USA) and Toronto (Canada) (Wong and Hanner 2008). In Ireland, a substitution rate of 25% was revealed among cod and haddock products, and this increased to 82% among smoked fish samples (Miller and Mariani 2010). A similar substitution rate of close to 80% was also found in shark seafood products in Italy (Barbuto et al. 2010). According to Caddy and Garibaldi (2000) only 65% of worldwide fishery captures reported to FAO for the year 1996 were identified at the species level, ranging from about 90% in temperate areas to less than 40% in tropical regions. High levels of substitution occur due to insufficient identification and inspection capacities, and can lead to misrepresentation of sustainable fisheries, such as those certified by the Marine Stewardship Council (Jacquet et al. 2010). This situation exposes retailers and consumers to cases of fraud, and poses a health risk in certain cases, such as those involving puffer fish (Cohen et al. 2009) and escolar (Lowenstein et al. 2009). Not only does this situation undermine consumer confidence in fish and seafood products, it places honest local or domestic producers at a disadvantage when fraudulent suppliers or importers undercut their margins through substitution, overfishing, or disobedience of fisheries regulations. Another prevalent concern is that mislabeling discounts the efforts of conscientious consumers in upholding conservation prohibitions (Logan et al. 2008; Wong and Hanner 2008).

DNA barcoding is a molecular method that utilizes the mitochondrial 5′ region of the cytochrome *c* oxidase subunit I (*COI*) gene for animal identification (Hebert et al. 2003). The method has been used very successfully to discriminate both marine fishes (e.g. Ward et al. 2005) and freshwater fishes (e.g. Hubert et al. 2008). The need for comprehensive and reliable species identification tools combined with early barcoding success among taxonomically diverse fishes led to the foundation of the Fish Barcode of Life (FISH-BOL) initiative (http://www.fishbol.org). FISH-BOL has the primary goal of gathering DNA barcode records for all the world's fishes, about 31,000 species (Ward et al. 2009). By April 2011 more than 8100 species have been barcoded, with greater than 25% coverage reached (Becker et al. 2011). The Barcode of Life Data Systems (BOLD, www.boldsystems.org; Ratnasingham and Hebert 2007), adopted by FISH-BOL, provides a sophisticated platform for DNA barcode data storage, management, and includes species identification tools.

DNA barcoding can be used to identify specimens including whole fish, fillets, fins, juveniles, larvae, eggs, or tissue fragments. It is recognized by the Food and Drug Administration in the USA as a replacement for the time-consuming technique of protein isoelectric focusing for fish identification (Yancy et al. 2008; Handy et al. 2011) and can be applied to raw, cooked (Wong and Hanner 2008), or smoked fish (Smith et al. 2008; Miller and Mariani 2010). It also has the potential to be used with heavily processed food samples by using short mini barcode regions (reviewed in Rasmussen et al. (2009)), and the reference library aids construction of molecular probes based on short, species-specific patterns of variation found in the standard barcode sequence (Eytan and Hellberg 2010; Rasmussen et al. 2010). The potential of DNA barcoding to provide unequivocal species assignments from whole or partial specimens may significantly reform seafood market practices, particularly for commercially important species (Rasmussen et al. 2009).

The objective of the present study was to assess the extent to which market names conform to the accepted trade names for seafood [as established by the Canadian Food Inspection Agency (CFIA)] by analyzing the results of several *ad hoc* surveys conducted in collaboration with various media outlets across Canada between 2008 and 2010. Given an estimated annual impact of US$240 billion from fisheries world-wide (Dyck and Sumaila 2010), the socioeconomic impact of seafood fraud deserves both public exposure and scientific documentation. Scientific names were obtained by matching specimen barcodes against reference sequence libraries from BOLD and GenBank. These were compared with the relevant species name(s) corresponding to the recorded market name as derived from the CFIA Fish List (http://www.inspection.gc.ca/english/fssa/fispoi/product/comnome.shtml).

Materials and methods

Seafood samples

A total of 254 seafood samples were purchased from various retailers, takeouts, and restaurants from 2008 to 2010 from five Canadian metropolitan areas, including Vancouver, Toronto, Gatineau, Montreal, and Quebec. Sample acquisition strategies for fillets generally targeted broad taxonomic coverage and diverse vendor coverage within each area, while sampling from restaurants and takeouts often targeted species known to be commonly substituted. Specimens were purchased, with tissues subsampled for analysis and preserved by freezing. All specimen provenance data, including the market name, were recorded locally. This work was carried out by investigative reporters from the Canadian Broadcasting Corporation (CBC Marketplace), Radio Canada (L'épicerie TV show), *The Vancouver Sun* newspaper, Montreal CTV TV station, and *The Toronto Star* newspaper. Tissues were then repacked in neutral packaging material and labeled with a neutral sample ID only

and shipped frozen to the Canadian Centre for DNA Barcoding for sample-blind molecular forensic analysis. A detailed overview of the samples is given in Table I.

DNA extraction

Upon arrival at the Canadian Centre for DNA Barcoding, frozen market samples were subsampled in a sterile flow hood. About 2 mm^3 tissue was extracted from the inside of each frozen sample, using tools that were treated with ELIMINase (Decon Laboratories, USA) between subsampling of different specimens. Each tissue piece was placed in a single-plate well with 30 µl of 95% ethanol for preservation. Genomic DNA was subsequently extracted with a membrane-based approach on a Biomek FX liquid handling station (Beckman Coulter, USA) using AcroPrep 96 1.0 ml filter plates with 1.0 µm PALL glass fiber media (Ivanova et al. 2006). To be able to generate a barcode from as many specimens as possible, 46 fillet samples that failed to generate a barcode after a first round of standard PCR and sequencing were later subsampled again as described above, and genomic DNA from these samples was extracted manually using the DNeasy Blood and Tissue kit (Qiagen, USA). In this manual processing, the tissue pieces were first incubated (under constant shaking at 300 rpm) overnight at 56°C in 180 µl tissue lysis buffer ATL and 20 µl proteinase K, followed by DNA extraction and final elution in 200 µl elution buffer, according to the manual of the Qiagen kit.

PCR amplification and sequencing

A 652 bp fragment of the standard COI DNA barcode region was amplified using either a fish and/or mammal PCR primer cocktail appended with M13 (Messing 1983) tails to aid in a standard sequencing protocol (Table II); see Ivanova et al. (2007) for details. The first PCR round was carried out with the fish PCR primer cocktail. A second attempt with the mammal cocktail was undertaken when the first attempt at PCR did not result in successful amplification or sequencing. When this approach was unsuccessful, genomic DNA was re-extracted manually from the samples affected (see above), and PCR was then repeated with the two primer cocktails described above. Each PCR mixture consisted of 6.25 µl of 10% trehalose, 2 µl of ultrapure ddH_2O, 1.25 µl of 10 × PCR buffer for Platinum *Taq* (Invitrogen, Inc., USA), 0.625 µl of 50 mM $MgCl_2$, 0.125 µl of 10 µM primer cocktail (see Table II), 0.0625 µl of 10 mM dNTP mix, 0.06 µl of Platinum *Taq* polymerase (Invitrogen), and 2.0 µl of template DNA in 12.5 µl of total reaction volume. PCR amplification reactions were conducted on Eppendorf Mastercycler *ep* gradient thermal cyclers (Brinkmann Instruments, USA). The thermocycling program consisted of a hot start of 94°C for 1 min; followed by five cycles of 94°C for 30 s, 50°C for 40 s, 72°C for 1 min; then 35 cycles of 94°C for 30 s, 54°C for 40 s, 72°C for 1 min; then an extension of 72°C for 10 min, and finally held at 4°C. PCR products were visualized on 2% agarose E-gel 96 plates (Invitrogen) stained with ethidium bromide. PCR samples with an abundant single band were bi-directionally sequenced using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems Inc. (ABI), USA). Each forward or reverse cycle sequencing reaction mixture consisted of 0.25 µl of BigDye (Applied Biosystems Inc.), 1.875 µl of 5 × buffer (400 mM Tris–HCl, pH 9.0, 10 mM $MgCl_2$), 5 µl of 10% trehalose, 1.0 µl of primer (10 µM; M13F or M13R, Table II), 0.875 µl of ultrapure ddH_2O, and 1.5 µl of PCR product. The sequencing reaction thermocycling program consisted of 2 min at 96°C, followed by 30 cycles of 30 s at 96°C, 15 s at 55°C, and 4 min at 60°C, followed by a hold at 4°C. Fluorescent signals were recorded on an ABI 3730 DNA analyzer.

Data evaluation and interpretation

Bi-directional sequences were assembled and edited using CodonCode Aligner software (CodonCode Corporation, USA). All sequence contig assemblies are provided in the Supplementary Figure S1. We analyzed the DNA barcode sequences derived from unknown samples with the "species"-level identification function of the BOLD ID Engine (version April 2011). A top species match was identified with a sequence similarity of at least 99%, and the results were double-checked via BLAST searches of the GenBank database. Species identifications for each specimen were compared with the relevant species name(s) corresponding to the recorded market name as derived from the CFIA Fish List (http://www.inspection.gc.ca/english/fssa/fispoi/product/comnome.shtml) (version April 2011). The criterion for the identification of potentially mislabeled samples was based solely on the literal information of acceptable species that can be sold under a given common market name (based on a strict interpretation of the CFIA Fish List) versus the name of the species inferred by barcoding. Therefore, some cases of mislabeled fish are potentially less egregious than others, and might not be considered surprising given common consumer knowledge and expectations. However, this literal approach was used to ensure that the determination of potential mislabeling was conducted consistently for all samples, which was particularly important in cases where a single market name is applied to multiple species, or multiple names across various name categories existed for a species (e.g. common, market, and vernacular names). An example of mislabeling is "Pacific Salmon",

Table I. Sample overview.

BOLD process ID	Sample name (label/menu)	Additional label info, fresh/frozen	Country of origin (label)	Country processed (label)	Price (CAN$) /kg	Species match in BOLD database	Similarity with BOLD match (%)	Unique species match	Mislabeled
Toronto, fillets									
CBCMS044-10	Mackerel	Peppered, smoked, frozen	UK	UK	22.45	*Scomber scombrus*	100.0	×	No
CBCMS045-10	Coho Salmon	Sliced, smoked, frozen	u* (Pacific)	Canada	19.49	*Oncorhynchus kisutch*	100.0	×	No
CBCMS046-10	Albacore Tuna	Wild, frozen	u	Canada	28.57	*Thunnus* sp.	100.0	×	Unresolved
CBCMS047-10	Haddock	Fillets, frozen	u	Scotland	34.95	*Melanogrammus aeglefinus*	100.0	×	No
CBCMS048-10	Rainbow Trout	Peppered, smoked, fillets, frozen	u	Norway	32.11	*Oncorhynchus mykiss*	100.0	×	No
CBCMS049-10	Shark	Steak, fresh	u	u	17.60	*Carcharhinus plumbeus*	100.0		Unresolved
CBCMS050-10	Sea Bass	Fillets, fresh	Chile	u	39.70	*Dissostichus eleginoides*	100.0	×	Yes (Patagonian Toothfish)
CBCMS053-10	Sockeye Salmon	Smoked, wild, frozen	u	u	70.47	*Oncorhynchus nerka*	100.0	×	No
CBCMS054-10	Ocean Perch	Fillets, frozen	u	China	14.98	*Sebastes* sp. (five species)†	100.0		No
CBCMS055-10	Pacific Pink Salmon	Wild, frozen	u	China	11.74	*Oncorhynchus gorbuscha*	100.0	×	No
CBCMS056-10	Halibut	Wild, steaks, frozen	USA (Alaska)	USA	u	*Hippoglossus stenolepis*	99.8	×	No
CBCMS057-10	Boston Bluefish	Fillets, frozen	u	Canada	11.23	*Pollachius virens*	100.0	×	No
CBCMS058-10	Pacific Salmon	Portions, frozen	u	China	14.98	*Oncorhynchus keta*	100.0	×	Yes (must include species)
CBCMS059-10	Pacific Salmon	Wild, smoked, fresh	u	Canada	28.63	*Oncorhynchus keta*	100.0	×	Yes (must include species)
CBCMS060-10	Pacific Salmon	Wild, fillets, frozen	u	u	25.08	*Oncorhynchus gorbuscha*	100.0	×	Yes (must include species)
CBCMS061-10	Halibut	Fillets, fresh	u (Pacific)	u	28.60	*Hippoglossus stenolepis*	100.0	×	No
CBCMS062-10	Steelhead Salmon	Fillet, fresh	u	u	22.02	*Oncorhynchus mykiss*	100.0	×	No
CBCMS063-10	Sea Bass	Fillet, fresh	Chile	u	44.10	*Dissostichus eleginoides*	100.0	×	Yes (Patagonian Toothfish)
CBCMS064-10	Orange Roughy	Fillet, frozen	u	u	28.64	*Hoplostethus atlanticus*	100.0	×	No
CBCMS065-10	Salmon	Farmed, organic, fillet, fresh	Ireland	u	26.43	*Salmo salar*	99.7	×	No
CBCMS066-10	Halibut	Wild, fillet, fresh	USA	u	26.43	*Hippoglossus stenolepis*	100.0	×	No
CBCMS067-10	Pacific Salmon	Wild, fillets, frozen	u	China	u	*Oncorhynchus gorbuscha*	100.0	×	Yes (must include species)
CBCMS068-10	Steelhead Trout	Cold smoked, frozen	u	u	u	*Oncorhynchus mykiss*	100.0	×	No
CBCMS069-10	Yellowfin Tuna	Wild caught, steaks, frozen	u	Vietnam	u	*Thunnus* sp.	100.0		Unresolved

Table I – *continued*

BOLD process ID	Sample name (label/menu)	Additional label info, fresh/frozen	Country of origin (label)	Country processed (label)	Price (CAN$) /kg	Species match in BOLD database	Similarity with BOLD match (%)	Unique species match	Mislabeled
CBCMS070-10	Basa	Fillets, frozen	u	u	8.80	*Pangasianodon hypophthalmus*	100.0	×	No
CBCMS071-10	Sockeye Salmon	Wild, smoked, frozen	USA (Alaska, Pacific)	Canada	70.47	*Oncorhynchus nerka*	100.0	×	No
CBCMS072-10	Pacific Salmon	Wild, frozen	USA (Alaska)	China	11.01	*Oncorhynchus gorbuscha*	99.9	×	Yes (must include species)
CBCMS073-10	Halibut	Wild, fresh	Canada (Atlantic)	u	44.07	*Hippoglossus stenolepis*	100.0	×	No
CBCMS074-10	Monkfish	Wild, fresh	USA/Canada	u	28.64	*Lophius americanus*	100.0	×	No
CBCMS075-10	Arctic Char	Farmed, fresh	Iceland	u	33.05	*Salvelinus alpinus*	100.0	×	No
CBCMS076-10	Snapper	Wild, j-cut, fresh	USA	u	19.82	*Sebastes flavidus*	100.0	×	Yes (Pacific Snapper)
CBCMS077-10	Turbot	Wild, fillet, fresh	USA (Boston)	u	22.02	*Reinhardtius hippoglossoides*	100.0	×	No
CBCMS078-10	Pacific Salmon	Wild, frozen	u	China	15.71	*Oncorhynchus gorbuscha*	100.0	×	Yes (must include species)
CBCMS079-10	Basa	Fillet, frozen	u	Vietnam	16.23	*Pangasianodon hypophthalmus*	99.9	×	No
CBCMS080-10	Albacore Tuna	Wild, frozen	u (Pacific)	Canada	28.57	*Thunnus* sp.	100.0		Unresolved
CBCMS081-10	Halibut	Wild, fillets, fresh	USA (Pacific)	u	44.07	*Hippoglossus stenolepis*	99.9	×	No
CBCMS082-10	Arctic Char	Fillets, fresh, farmed	Iceland	u	33.05	*Salvelinus alpinus*	100.0	×	No
CBCMS083-10	Monkfish	Wild, fillet, fresh	USA/Canada	u	28.64	*Lophius americanus*	100.0	×	No
CBCMS084-10	Ocean Perch	Fresh	Canada (Atlantic)	u	19.81	*Sebastes* sp. (five species)†	100.0		No
CBCMS085-10	Pickerel	Wild, fillets, fresh	USA/Canada	u	28.64	*Sander vitreus*	100.0	×	No
CBCMS086-10	Snapper	Wild, j-cut, fillet, fresh	USA	u	19.82	*Sebastes entomelas*	99.8	×	Yes (Widow Rockfish)
CBCMS087-10	Halibut	Wild, frozen	u (Pacific)	u	42.86	*Hippoglossus stenolepis*	99.7	×	No
CBCMS088-10	Coho Salmon	Wild, fresh	Canada (BC)	u	26.60	*Salmo salar*	100.0	×	Yes (Atlantic Salmon, or salmon)
CBCMS089-10	Sole	Fresh, fillet, fresh	u	u	22.02	*Microstomus pacificus*	99.9	×	No
CBCMS090-10	Halibut	Fresh	Chile	u	37.46	*Hippoglossus stenolepis*	100.0	×	No
CBCMS091-10	Red Spring Salmon	Wild, fresh	BC	u	28.60	*Salmo salar*	100.0	×	Yes (Atlantic Salmon, or salmon)
CBCMS163-10	Ocean Perch	Fillets, fresh	u	u	17.61	*Sebastes* sp. (five species)†	100.0		No
Vancouver fillets									
CBCMS001-10	Basa	Fillet, frozen	Vietnam	u	17.90	*Pangasianodon hypophthalmus*	100.0	×	No

Table I – *continued*

BOLD process ID	Sample name (label/menu)	Additional label info, fresh/frozen	Country of origin (label)	Country processed (label)	Price (CAN$) /kg	Species match in BOLD database	Similarity with BOLD match (%)	Unique species match	Mislabeled
CBCMS002-10	Red Snapper	Fresh	Canada	u	23.90	*Sebastes entomelas*	99.8	×	Yes (Widow Rockfish)
CBCMS003-10	Halibut	Frozen	Canada	u	43.90	*Hippoglossus stenolepis*	99.9	×	No
CBCMS004-10	Salmon	Wild, frozen	Canada	u	32.90	*Oncorhynchus nerka*	100.0	×	Yes (must include species)
CBCMS005-10	Sole	Fillet, fresh	Canada	u	23.90	*Microstomus pacificus*	100.0	×	No
CBCMS006-10	Basa	Frozen	Vietnam	u	17.90	*Pangasianodon hypophthalmus*	100.0	×	No
CBCMS007-10	Atlantic Salmon	Fresh	Canada (Atlantic)	u	32.90	*Salmo salar*	99.9	×	No
CBCMS008-10	Tuna Ahi	Fresh	u	u	64.90	*Thunnus* sp.	100.0		Unresolved
CBCMS009-10	Halibut	Frozen	Pacific	u	49.90	*Hippoglossus stenolepis*	99.9	×	No
CBCMS010-10	Sockeye	Wild, frozen	Pacific	u	32.90	*Oncorhynchus nerka*	100.0	×	No
CBCMS011-10	Red Snapper	Fresh	Canada	u	23.90	*Sebastes flavidus*	100.0	×	Yes (Pacific Snapper)
CBCMS012-10	Atlantic Salmon	Fresh	Canada (Atlantic)	u	32.90	*Salmo salar*	100.0	×	No
CBCMS013-10	Basa	Fillet, frozen	Vietnam	u	17.90	*Pangasianodon hypophthalmus*	100.0	×	No
CBCMS014-10	Barramundi	Frozen	Australia	USA	29.38	*Lates calcarifer*	100.0	×	No
CBCMS015-10	Capensis	Wild, frozen	u	u	11.75	*Merluccius capensis*	100.0	×	No
CBCMS016-10	Halibut	Frozen	u	u	33.90	*Hippoglossus stenolepis*	99.7	×	No
CBCMS017-10	Mackerel	Smoked, fresh	Canada	u	29.90	*Scomber scombrus*	100.0	×	No
CBCMS018-10	Black Cod	Wild, frozen	Canada	u	46.39	*Anoplopoma fimbria*	99.9	×	No
CBCMS019-10	Mahi-Mahi	Frozen	u	u	25.00	*Coryphaena hippurus*	99.9	×	No
CBCMS020-10	Rainbow Trout	Fresh	Canada	u	16.90	*Oncorhynchus mykiss*	100.0	×	No
CBCMS021-10	Red Snapper	Fresh	Pacific	u	17.90	*Sebastes alutus*	100.0	×	Yes
CBCMS022-10	Sockeye Salmon	Wild, frozen	USA (Alaska)	u	19.90	*Oncorhynchus nerka*	100.0	×	No
CBCMS023-10	Albacore Tuna	Wild, frozen	u	u	28.57	*Thunnus* sp.	100.0		Unresolved
CBCMS024-10	Atlantic Salmon	Fresh, farmed	u (Atlantic)	u	18.90	*Salmo salar*	100.0	×	No
CBCMS025-10	Sockeye Salmon	Frozen	USA (Alaska)	u	19.90	*Oncorhynchus nerka*	100.0	×	No
CBCMS026-10	Red Snapper	Fresh	u (Pacific)	u	17.90	*Sebastes alutus*	100.0	×	Yes (four other names)‡
CBCMS027-10	Halibut	Frozen	USA (Alaska)	u	31.90	*Hippoglossus stenolepis*	100.0	×	No
CBCMS028-10	Sole	Fillet, fresh	u (Pacific)	u	18.90	*Microstomus pacificus*	100.0	×	No
CBCMS029-10	Red Snapper	Fresh	u	u	16.90	*Sebastes flavidus*	100.0	×	Yes (Pacific Snapper)
CBCMS030-10	Cat Fish	Fresh	u	u	25.90	*Ictalurus punctatus*	100.0	×	No
CBCMS031-10	Halibut	Frozen	u	u	33.90	*Hippoglossus stenolepis*	100.0	×	No
CBCMS032-10	True Cod	Fresh	u	u	28.90	*Gadus macrocephalus*	99.9	×	No
CBCMS033-10	Sockeye Salmon	Wild, frozen	u	u	22.90	*Oncorhynchus nerka*	100.0	×	No
CBCMS034-10	Steelhead Trout	Fresh	Chile	u	22.00	*Oncorhynchus mykiss*	100.0	×	No
CBCMS035-10	Basa	Fillets, frozen	Vietnam	u	10.30	*Pangasianodon hypophthalmus*	100.0	×	No

Table I – *continued*

BOLD process ID	Sample name (label/menu)	Additional label info, fresh/frozen	Country of origin (label)	Country processed (label)	Price (CAN$) /kg	Species match in BOLD database	Similarity with BOLD match (%)	Unique species match	Mislabeled
CBCMS036-10	Mako Shark	Fresh	USA (Florida)/Cuba	u	29.00	*Isurus oxyrinchus*	100.0	×	No
CBCMS037-10	Sword Fish	Fresh	USA (Florida)/Cuba	u	38.90	*Xiphias gladius*	100.0	×	No
CBCMS038-10	Blue Marlin	Fresh	USA (Florida)/Cuba	u	38.92	*Makaira nigricans*	100.0	×	No
CBCMS039-10	Arctic Char	Fresh	USA (Alaska)	u	39.00	*Salvelinus* sp.	100.0		Unresolved
CBCMS040-10	Halibut	Fillet, frozen	(Pacific)	u	33.90	*Hippoglossus stenolepis*	100.0	×	No
CBCMS041-10	Red Snapper	Fresh	u	u	16.90	*Sebastes flavidus*	100.0	×	Yes (Pacific Snapper)
CBCMS042-10	Sockeye Salmon	Wild, fillet, frozen	(Pacific)	u	22.90	*Oncorhynchus nerka*	100.0	×	No
CBCMS043-10	Cod	Fillet, counter, fresh	(Pacific)	u	20.90	*Gadus macrocephalus*	100.0	×	No
Quebec, fillets									
CBCMS092-10	Sole	Fillets, fresh	u (Pacific)	u	16.51	*Microstomus pacificus*	100.0	×	No
CBCMS094-10	Tuna	Loins, regular, fresh	u	u	39.66	*Thunnus* sp.	100.0		No
CBCMS095-10	Blue Marlin	Steaks, fresh	u	u	25.33	*Makaira nigricans*	100.0	×	No
CBCMS096-10	Sockeye Salmon	Smoked, fresh	u	u	59.00	*Oncorhynchus nerka*	100.0	×	No
CBCMS097-10	Halibut	Steaks, frozen	u (Pacific)	u	35.25	*Hippoglossus hippoglossus*	100.0	×	No
CBCMS098-10	Tuna	Loin, steaks, grade 1, fresh	u	u	39.66	*Thunnus* sp.	100.0		No
CBCMS099-10	Pickerel	Boned, fillets, fresh	u	u	30.84	*Sander vitreus*	99.9	×	No
CBCMS100-10	Cod	Skinless, boneless, fillets, frozen	u	China	14.98	*Gadus macrocephalus*	99.9	×	No
CBCMS101-10	Sockeye Salmon	Wild, smoked, frozen	u (Pacific)	u	70.47	*Oncorhynchus nerka*	100.0	×	No
CBCMS102-10	Coho Salmon	Wild, smoked, frozen	u (Pacific)	u	74.90	*Oncorhynchus kisutch*	100.0	×	No
CBCMS103-10	Sockeye Salmon	Wild, smoked, frozen	u (Pacific)	u	94.00	*Oncorhynchus nerka*	100.0	×	No
CBCMS104-10	Pacific Salmon	Portions, frozen	u	China	9.98	*Oncorhynchus keta*	100.0	×	Yes (must include species)
CBCMS105-10	Yellowfin Tuna	Steaks, frozen	u	Vietnam	15.40	*Thunnus* sp.	100.0		Unresolved
CBCMS106-10	Albacore Tuna	Wild, smoked, frozen	u	u	71.25	*Thunnus* sp.	100.0		Unresolved
CBCMS107-10	Halibut	Wild, smoked, frozen	USA (Alaska)	u	89.11	*Hippoglossus stenolepis*	99.9	×	No
CBCMS108-10	Atlantic Cod	Fillets, fresh	u	u	15.41	*Gadus chalcogrammus*	99.9	×	Yes (pollock)
CBCMS109-10	Atlantic Salmon	Fresh	u	u	28.50	*Salmo salar*	100.0	×	No
CBCMS110-10	Pacific Salmon	Frozen	u	China	12.50	*Oncorhynchus keta*	99.5	×	Yes (must include species)
CBCMS111-10	Cod	Fillets, frozen	u	China	14.69	*Gadus macrocephalus*	100.0	×	No

Table I – *continued*

BOLD process ID	Sample name (label/menu)	Additional label info, fresh/frozen	Country of origin (label)	Country processed (label)	Price (CAN$) /kg	Species match in BOLD database	Similarity with BOLD match (%)	Unique species match	Mislabeled
CBCMS112-10	Pacific Salmon	Wild, frozen	u	China	14.96	*Oncorhynchus gorbuscha*	100.0	×	Yes (must include species)
CBCMS113-10	Sockeye Salmon	Wild, smoked, frozen	u	China	87.72	*Oncorhynchus nerka*	100.0	×	No
CBCMS114-10	Atlantic Salmon	Boneless, fresh	u	u	17.61	*Salmo salar*	100.0	×	No
CBCMS115-10	Tilapia	Boneless, fillets, fresh	u	u	18.72	*Oreochromis niloticus*	100.0	×	No
Gatineau, fillets									
CBCMS116-10	Chilean Sea Bass	Frozen	u	Chile	44.07	*Dissostichus eleginoides*	100.0	×	Yes (Patagonian Toothfish)
CBCMS117-10	Tuna	Loin, regular, fresh	u	u	39.66	*Thunnus* sp.	99.9		No
CBCMS118-10	Orange Ruffy	Fillets, frozen	u	u	27.99	*Hoplostethus atlanticus*	99.8	×	No (Orange Roughy—common misspelling)
CBCMS120-10	Pickerel	Wild, fresh	Canada	u	28.64	*Sander vitreus*	100.0	×	No
CBCMS121-10	Cod	Large fillets, fresh	u	u	20.92	*Melanogrammus aeglefinus*	100.0	×	Yes (haddock)
CBCMS122-10	Yellowfin Tuna	Smoked, frozen	u	Canada	64.21	*Thunnus* sp.	100.0		Unresolved
CBCMS124-10	Tuna	Steak, fresh	u	u	44.06	*Thunnus* sp.	100.0		No
CBCMS125-10	Pacific Salmon	Wild, frozen	u	China	19.90	*Oncorhynchus keta*	100.0	×	Yes (must include species)
Montreal, fillets									
CBCMS126-10	Atlantic Salmon	Frozen	Chile	Chile	17.61	*Salmo salar*	99.7	×	No
CBCMS128-10	Tuna	Loin, regular, fresh	u	u	39.66	*Thunnus* sp.	100.0		No
CBCMS129-10	Salmon	Meat, frozen	u	u	10.50	*Salmo salar*	100.0	×	No
CBCMS130-10	Blue Marlin	Steaks, fresh	u	u	25.33	*Makaira nigricans*	100.0	×	No
CBCMS131-10	Chilean Sea Bass	Fresh	u	u	55.09	*Dissostichus eleginoides*	100.0	×	Yes (Patagonian Toothfish)
CBCMS132-10	Tuna	Loin, regular, fresh	u	u	39.66	*Thunnus* sp.	100.0		No
CBCMS134-10	Arctic Char	Fillets, fresh	u	u	33.05	*Salvelinus* sp.	100.0		Unresolved
CBCMS135-10	Blue Marlin	Steaks, fresh	u	u	24.23	*Makaira nigricans*	100.0	×	No
CBCMS136-10	Pacific Snapper	Fillets, fresh	u	u	17.61	*Sebastes flavidus*	100.0	×	No
CBCMS137-10	Cod	Fillets, fresh	u	u	20.92	*Melanogrammus aeglefinus*	100.0	×	Yes (haddock)
CBCMS138-10	Tuna	Loin, sashimi grade, fresh	u	u	55.09	*Thunnus* sp.	100.0		No
CBCMS139-10	Blue Marlin	Steaks, fresh	u	u	24.23	*Makaira nigricans*	100.0	×	No
CBCMS140-10	Pacific Snapper	Fillets, fresh	u	u	17.61	*Sebastes flavidus*	100.0	×	No
CBCMS141-10	Blue Marlin	Steaks, fresh	u	u	24.23	*Makaira nigricans*	100.0	×	No
CBCMS142-10	Cod	Fillets, fresh	u	u	20.92	*Melanogrammus aeglefinus*	100.0	×	Yes (haddock)

Table I – *continued*

BOLD process ID	Sample name (label/menu)	Additional label info, fresh/frozen	Country of origin (label)	Country processed (label)	Price (CAN$) /kg	Species match in BOLD database	Similarity with BOLD match (%)	Unique species match	Mislabeled
CBCMS143-10	Pacific Snapper	Fillets, fresh	u	u	16.51	*Sebastes entomelas*	99.8	×	Yes (Widow Rockfish)
CBCMS144-10	Tuna	Loin, sashimi grade, fresh	u	u	55.09	*Thunnus* sp.	100.0		No
CBCMS145-10	Atlantic Salmon	Fillets, fresh	u	u	35.25	*Salmo salar*	100.0	×	No
CBCMS146-10	Tuna	Steak, fresh	u	u	44.06	*Thunnus* sp.	100.0		No
CBCMS147-10	Sole	Fillets, fresh	u (Pacific)	u	17.61	*Microstomus pacificus*	100.0	×	No
CBCMS148-10	Alaskan Halibut	Wild, smoked, frozen	USA (Alaska)	u	89.11	*Hippoglossus stenolepis*	100.0	×	No
CBCMS149-10	Tuna	Steak, fresh	u	u	44.06	*Thunnus* sp.	100.0		No
CBCMS150-10	Blue Marlin	Loin, fresh	u	u	28.64	*Makaira nigricans*	100.0	×	No
CBCMS151-10	Steelhead Salmon	Smoked, frozen	u	Norway	57.38	*Oncorhynchus kisutch*	100.0	×	Yes (Coho Salmon)
CBCMS153-10	Chilean Sea Bass	Fresh	u	Chile	48.48	*Dissostichus eleginoides*	100.0	×	Yes (Patagonian Toothfish)
CBCMS154-10	Black Cod	Fresh	u	u	48.47	*Anoplopoma fimbria*	100.0	×	No
CBCMS155-10	Arctic Char	Fillets, fresh	u	u	33.05	*Salvelinus* sp.	100.0		Unresolved
CBCMS156-10	Blue Marlin	Fresh	u	u	24.22	*Makaira nigricans*	99.9	×	No
CBCMS157-10	Pacific Halibut	Steaks, fresh	u	u	37.46	*Hippoglossus hippoglossus*	99.9	×	Yes (Atlantic Halibut, or Halibut)
CBCMS158-10	Pacific Salmon	Wild, frozen	u	u	8.79	*Oncorhynchus keta*	100.0	×	Yes (must include species)
CBCMS159-10	Yellowfin Tuna	Fresh	u	u	61.62	*Thunnus* sp.	100.0		Unresolved
CBCMS160-10	Atlantic Halibut	Steak, fresh	u	u	48.39	*Hippoglossus hippoglossus*	99.8	×	No
CBCMS161-10	Pacific Salmon	Wild, fresh	u	u	26.00	*Salmo salar*	100.0	×	Yes (Atlantic Salmon, or Salmon)
CBCMS162-10	Tuna	Regular, fresh	u	u	55.00	*Thunnus* sp.	100.0		No
Vancouver takeout, sushi									
LPVMS01-09	Cod	Deep fried	u	u	u	*Gadus macrocephalus*	100.0	×	No
LPVMS02-09	Halibut	Deep fried	u	u	u	*Hippoglossus stenolepis*	100.0	×	No
LPVMS03-09	Cod	u	u	u	u	*Gadus chalcogrammus*	100.0	×	Yes (Pollock)
LPVMS04-09	Halibut	u	u	u	u	*Hippoglossus stenolepis*	99.7	×	No
LPVMS05-09	White Tuna	u	u	u	u	*Thunnus* sp.	100.0		Unresolved
LPVMS06-09	Sockeye Salmon	Wild	u	u	u	*Oncorhynchus nerka*	100.0	×	No
LPVMS07-09	Snow Crab	u	u	u	u	*Chionoecetes opilio*	99.7	×	No
LPVMS08-09	Yellowfin Tuna	u	u	u	u	*Seriola quinqueradiata*	100.0	×	Yes (Japanese Amberjack)
LPVMS09-09	Red Snapper	Fillet	u	u	19.80	*Sebastes ruberrimus*	100.0	×	No
LPVMS10-09	Rock Cod	Fillet	u	u	15.40	*Sebastes alutus*	100.0	×	Yes (Rockfish)

Table I – *continued*

BOLD process ID	Sample name (label/menu)	Additional label info, fresh/frozen	Country of origin (label)	Country processed (label)	Price (CAN$) /kg	Species match in BOLD database	Similarity with BOLD match (%)	Unique species match	Mislabeled
LPVMS13-09	Red Snapper	Deep fried	u	u	u	*Sebastes* sp.	99.8		Unresolved
LPVMS14-09	Cod	Deep fried	u	u	u	*Micromesistius australis*	100.0	×	Yes (Blue Cod)
LPVMS15-09	Sockeye	Wild, Sushi	u	u	u	*Oncorhynchus nerka*	100.0	×	No
LPVMS16-09	Halibut	Sushi	u	u	u	*Paralichthys olivaceus*	99.8	×	Yes (not on CFIA list; is Bastard Halibut)
LPVMS19-09	Fish	Fillet, Sandwich	u	u	u	*Gadus chalcogrammus*	100.0	×	Yes (pollock)
LPVMS20-09	Red Snapper	Fillet, fresh	u	u	19.80	*Sebastes babcocki*	100.0	×	Yes (Pacific Snapper)
LPVMS21-09	Cod	Fillet, previously frozen	u	u	24.21	*Gadus macrocephalus*	100.0	×	No
LPVMS22-09	Cod	Deep fried	u	u	u	*Gadus macrocephalus*	100.0	×	No
LPVMS27-09	Halibut	Deep fried	u	u	u	*Hippoglossus stenolepis*	100.0	×	No
LPVMS28-09	Cod	Deep fried	u	u	u	*Pangasianodon hypophthalmus*	100.0	×	Yes (basa)
LPVMS29-09	Cod	BC roll	u	u	u	*Merluccius productus*	100.0	×	Yes (Whiting, or Hake)
Montreal, sushi									
CTVM001-09	Red Snapper	u	u	u	u	*Oreochromis niloticus*	100.0		Yes (tilapia)
CTVM002-09	Tuna	u	u	u	u	*Thunnus obesus*	100.0	×	No
CTVM003-09	Salmon	u	u	u	u	*Oncorhynchus mykiss*	100.0	×	Yes (Steelhead Salmon)
CTVM004-09	Red Snapper	u	u	u	u	*Oreochromis niloticus*	100.0	×	Yes (tilapia)
CTVM005-09	White Tuna	u	u	u	u	*Thunnus* sp.	100.0		Unresolved
CTVM006-09	Salmon	u	u	u	u	*Salmo salar*	100.0	×	No
CTVM007-09	Cuttlefish	u	u	u	u	*Mallotus villosus*	100.0	×	Yes (caplin)
CTVM008-09	Yellowtail	u	u	u	u	*Seriola quinqueradiata*	100.0	×	Yes (Japanese Amberjack)
CTVM009-09	Flying Fish Roe	u	u	u	u	*Mallotus villosus*	100.0	×	Yes (caplin)
CTVM010-09	Red Tuna	u	u	u	u	*Thunnus obesus*	100.0	×	Yes (tuna)
CTVM011-09	Capelin Roe	u	u	u	u	*Mallotus villosus*	100.0	×	No
CTVM012-09	Salmon	u	u	u	u	*Salmo salar*	100.0	×	No
CTVM013-09	White Tuna	u	u	u	u	*Thunnus* sp.	100.0		Unresolved
CTVM014-09	Eel	u	u	u	u	*Anguilla japonica*	99.8	×	No
CTVM015-09	Red Snapper	u	u	u	u	*Oreochromis niloticus*	99.8	×	Yes (tilapia)
CTVM016-09	Red Snapper	u	u	u	u	*Oreochromis niloticus*	100.0	×	Yes (tilapia)
CTVM017-09	Mackerel	u	u	u	u	*Scomber scombrus*	100.0	×	No
CTVM018-09	Yellowtail	u	u	u	u	*Seriola lalandi*	100.0	×	Yes (Yellowtail Amberjack)
CTVM019-09	Red Tuna	u	u	u	u	*Thunnus obesus*	100.0	×	Yes (tuna)
CTVM020-09	Salmon	u	u	u	u	*Salmo salar*	100.0	×	No
CTVM021-09	Red Snapper	u	u	u	u	*Oreochromis niloticus*	100.0	×	Yes (tilapia)

Table I – *continued*

BOLD process ID	Sample name (label/menu)	Additional label info, fresh/frozen	Country of origin (label)	Country processed (label)	Price (CAN$) /kg	Species match in BOLD database	Similarity with BOLD match (%)	Unique species match	Mislabeled
CTVM022-09	Red Tuna	u	u	u	u	*Thunnus obesus*	100.0	×	Yes (tuna)
CTVM023-09	White Tuna	u	u	u	u	*Thunnus* sp.	100.0		Unresolved
CTVM024-09	Red Snapper	u	u	u	u	*Oreochromis aureus*	99.7	×	Yes (Blue Tilapia)
CTVM025-09	Striped Bass	u	u	u	u	*Morone chrysops*	100.0	×	Yes (White Bass)
CTVM026-09	Yellowtail	u	u	u	u	*Oreochromis niloticus*	100.0	×	Yes (tilapia)
CTVM027-09	Red Snapper	u	u	u	u	*Seriola quinqueradiata*	100.0	×	Yes (Japanese Amberjack)
CTVM028-09	Eel	u	u	u	u	*Anguilla japonica*	99.5	×	No
CTVM029-09	Red Snapper	u	u	u	u	*Oreochromis mossambicus*	100.0	×	Yes (Mozambique Tilapia)
CTVM030-09	Yellowtail	u	u	u	u	*Seriola quinqueradiata*	100.0	×	Yes (Japanese Amberjack)
CTVM031-09	Yellowtail	u	u	u	u	*Seriola quinqueradiata*	100.0	×	Yes (Japanese Amberjack)
CTVM032-09	Red Snapper	u	u	u	u	*Oreochromis niloticus*	100.0	×	Yes (tilapia)
CTVM033-09	Red Tuna	u	u	u	u	*Thunnus* sp.	100.0		Unresolved
CTVM034-09	White Tuna	u	u	u	u	*Oreochromis mossambicus*	100.0	×	Yes (Mozambique Tilapia)
CTVM035-09	Snapper	u	u	u	u	*Oreochromis niloticus*	100.0	×	Yes (tilapia)
CTVM037-09	Red Snapper	u	u	u	u	*Oreochromis urolepis hornorum*	100.0	×	Yes (Wami Tilapia)
CTVM038-09	Salmon	u	u	u	u	*Salmo salar*	100.0	×	No
CTVM039-09	Red Tuna	u	u	u	u	*Thunnus obesus*	100.0	×	Yes (tuna)
CTVM040-09	Yellowfin Tuna	u	u	u	u	*Thunnus* sp.	100.0		Unresolved
CTVM041-09	Red Snapper	u	u	u	u	*Oreochromis aureus*	99.7	×	Yes (tilapia)
CTVM042-09	Cobia	u	u	u	u	*Rachycentron canadum*	100.0	×	No
CTVM043-09	Flying Fish (caviar)	u	u	u	u	*Hirundichthys oxycephalus*	99.3	×	Yes (not on CFIA list; is Bony Flying Fish)
CTVM044-09	Whitefish	u	u	u	u	*Oreochromis niloticus*	100.0	×	Yes (tilapia)
CTVM045-09	Mackerel	u	u	u	u	*Scomber scombrus*	99.5	×	No
CTVM046-09	Flying Fish Roe	u	u	u	u	*Mallotus villosus*	100.0	×	Yes (caplin)
CTVM047-09	Red Tuna	u	u	u	u	*Thunnus obesus*	100.0	×	Yes (tuna)
CTVM048-09	Eel	u	u	u	u	*Anguilla japonica*	99.8	×	No
CTVM049-09	Red Snapper	u	u	u	u	*Oreochromis mossambicus*	100.0	×	Yes (Mozambique Tilapia)
Toronto, sushi									
SSTSS001-09	Red Snapper	u	u	u	u	No species match, genus *Oreochromis*			Yes (does not match any Red Snapper)

Table I – *continued*

BOLD process ID	Sample name (label/menu)	Additional label info, fresh/frozen	Country of origin (label)	Country processed (label)	Price (CAN$) /kg	Species match in BOLD database	Similarity with BOLD match (%)	Unique species match	Mislabeled
SSTSS002-09	Red Snapper	u	u	u	u	No species match			Yes (does not match any Red Snapper)
SSTSS003-09	Red Snapper	u	u	u	u	*Oreochromis niloticus*	99.9	×	Yes (tilapia)
SSTSS004-09	Snapper	u	u	u	u	No species match, genus *Oreochromis*			Yes (does not match any Snapper)
SSTSS005-09	Red Snapper	u	u	u	u	*Oreochromis aureus*	100.0	×	Yes (tilapia)
SSTSS006-09	Red Snapper	u	u	u	u	No species match			Yes (does not match any Red Snapper)
SSTSS007-09	Red Snapper	u	u	u	u	*Oreochromis niloticus*	100.0	×	Yes (tilapia)
SSTSS008-09	Red Snapper	u	u	u	u	No species match, genus *Oreochromis*			Yes (does not match any Red Snapper)
SSTSS009-09	Red Snapper	u	u	u	u	*Oreochromis niloticus*	100.0	×	Yes (tilapia)
SSTSS010-09	Red Snapper	u	u	u	u	*Pagrus major*	100.0	×	Yes (Silver, Japanese Seabream)
SSTSS011-09	Japanese Seabream	u	u	u	u	*Oreochromis niloticus*	100.0	×	Yes (tilapia)
SSTSS012-09	Red Snapper	u	u	u	u	No species match, genus *Oreochromis*			Yes (does not match any Red Snapper)

* u, unknown; † *Sebastes* sp. (*fasciatus*, *marinus*, *mentella*, *norvegicus*, *viviparus*); ‡ Rose Perch, Rock Perch, Redfish, or Pacific Ocean Perch.

Table II. PCR and sequencing primers used in the present study.

Name	Cocktail name/5′–3′ sequence	Reference
	M13-tailed primers	
	Fish: C_FishF1t1—C_FishR1t1 (ratio 1:1:1:1)	Ivanova et al. (2007)
VF2_t1	TGTAAAACGACGGCCAGTCAACCAACCACAAAGACATTGGCAC	
FishF2_t1	TGTAAAACGACGGCCAGTCGACTAATCATAAAGATATCGGCAC	
FishR2_t1	CAGGAAACAGCTATGACACTTCAGGGTGACCGAAGAATCAGAA	
FR1d_t1	CAGGAAACAGCTATGACACCTCAGGGTGTCCGAARAAYCARAA	
	Mammal: C_VF1LFt1—C_VR1LRt1 (ratio 1:1:1:3:1:1:1:3)	Ivanova et al. (2007)
LepF1_t1	TGTAAAACGACGGCCAGTATTCAACCAATCATAAAGATATTGG	
VF1_t1	TGTAAAACGACGGCCAGTTCTCAACCAACCACAAAGACATTGG	
VF1d_t1	TGTAAAACGACGGCCAGTTCTCAACCAACCACAARGAYATYGG	
VF1i_t1	TGTAAAACGACGGCCAGTTCTCAACCAACCAIAAIGAIATIGG	
LepR1_t1	CAGGAAACAGCTATGACTAAACTTCTGGATGTCCAAAAAATCA	
VR1d_t1	CAGGAAACAGCTATGACTAGACTTCTGGGTGGCCRAARAAYCA	
VR1_t1	CAGGAAACAGCTATGACTAGACTTCTGGGTGGCCAAAGAATCA	
VR1i_t1	CAGGAAACAGCTATGACTAGACTTCTGGGTGICCIAAIAAICA	
	Sequencing primers for M13-tailed PCR products	
M13F	TGTAAAACGACGGCCAGT	Messing (1983)
M13R	CAGGAAACAGCTATGAC	Messing (1983)

because in Canada all Pacific Salmon must include a species' common name (see: http://www.inspection.gc.ca/english/fssa/fispoi/commun/20101220e.shtml). Hence, irrespective of DNA testing, some specimens are mislabeled simply because the label failed to conform to an accepted common name. However, mislabeling also relates to market substitution in cases when an accepted common name (as indicated on the label or menu) failed to match the expected species identity as revealed by barcoding. Examples are the substitution of Red Snapper with Tilapia, or Coho Salmon with Atlantic Salmon. Thus, all cases of "substitution" are considered mislabeled (see Table I).

Results and discussion

For the present study, 254 fish and seafood samples were collected at retail outlets, takeouts, and restaurants in five Canadian metropolitan areas including Vancouver, Toronto, Gatineau, Montreal, and Quebec. Among these samples, 236 (93.3%) yielded high-quality sequences with a length of at least 418 bp. The observed failure rate of 6.7% (Table III) is similar to other fish barcoding studies (e.g. Ward et al. 2009; Nwani et al. 2011). For those samples that yielded a barcode, we used the BOLD "species-level" identification tool to query the sample barcode against the reference database (details in Materials and methods), which provided a sequence similarity value greater than 99% for 230 of the 236 samples with a barcode (Table I). The remaining six samples exhibited no significant match to anything in the database. Of the 230 samples with a match, 195 samples were unambiguously assignable to a single species (Table I). However, 35 samples were unresolved (e.g. sample CBCMS046-10 of *Thunnus* sp.) in cases where two or more sister species appear to share a barcode in the reference library. For example, the ability to discriminate closely related species of *Thunnus* is problematic (Chow and Kishino 1995); and this is true even for barcoding (Viñas and Tudela 2009; but see Lowenstein et al. 2009). Because different researchers have used different sets of reference sequences and because voucher specimens are lacking for these sequences, it is impossible to validate the identifications assigned to the reference

Table III. Summary results for 236 seafood samples barcoded (out of 254 samples submitted) from across Canada.

City	Samples (*n*), kind	Multiple matches	Barcode ID, *n* (%)	Mislabeled, *n* (%)⋆
Toronto	47, fillets	7	40 (85.1)	12 (28.0)
Vancouver	43, fillets	3	40 (93.0)	7 (17.5)
Quebec	23, fillets	4	19 (82.6)	4 (19.0)
Gatineau	8, fillets	3	5 (62.5)	3 (42.9)
Montreal	34, fillets	10	24 (70.6)	10 (32.3)
Vancouver	21, takeout, sushi	2	19 (90.5)	9 (47.4)
Montreal	48, sushi	6	42 (87.5)	31 (72.1)
Toronto	12, sushi	0†	6 (50.0)	12 (100.0)
All	236	35	195 (82.6)	88 (41.1)

⋆ In relation to number of samples with barcode (also if multiple matches are clearly different); † Six samples could not be assigned to a species using the BOLD species ID engine, although four of the six were close matches to various tilapia species in the genus *Oreochromis* and mislabeled.

sequences in question and thereby resolve conflicts within or between studies. Hence, some level of ambiguity exists in the accuracy of the underlying taxonomic identifications. Alternatively, closely related species may be in a state of incomplete lineage diversification where they retain ancestral polymorphisms, occasionally hybridize, or both. While this is the exception rather than the norm, certain taxonomically challenging species complexes involving genera such as *Salvelinus* and *Sebastes* include closely related sister species that appear to share barcodes. This is not surprising because hybridization or incomplete lineage sorting of ancestral polymorphisms has been documented previously in *Sebastes* (Steinke et al. 2009) and *Salvelinus* (Baxter et al. 1997). While identification could only be resolved to a congeneric species complex in such cases (see footnotes for Table I), this level of resolution was still sufficient to detect gross substitution (Table I).

Taxonomic misidentification of reference specimens complicates the resolution of apparent haplotype sharing, yet cleansing the reference database of such identification errors requires a concerted effort that often includes both the original specimen collector, and in some cases additional experts who can provide an independent identification of the specimens that represent outliers in the database. Most species are represented by a single, cohesive cluster of barcodes that do not overlap with any other named species (Table I) and these patterns are typically reinforced with the accumulation of additional reference sequences derived from independent sources. However, discrepancies do arise and annotating the library accordingly is an ongoing process. Besides, vetting voucher identifications, critical points for data curation include ranking taxonomic identifications using a quality metric (Steinke and Hanner 2011), as well as considering the proximity of barcoded specimens to the type locality of that species (Lowenstein et al. 2011) and flagging for removal from the ID engine those outliers that appear to be contaminants or whose identifications cannot be substantiated. The barcode-based identification of poorly sampled species represents a challenge for forensics (Wilson-Wilde et al. 2010), where interpretation of matches may require expert opinion. However, barcoding is making species-level identifications more accessible through BOLD, despite some conflicts. Indeed, the database has been proven very reliable in this study ($>99\%$ similarity for most samples tested; Table I). Ultimately, regulatory agencies require full transparency and traceability for any sequences that are used in regulatory decisions. Barcoding and BOLD support these objectives and provide a platform for data curation, but curatorial annotation practices must be implemented to circumscribe the expert interpretation of conflicting data. Otherwise, the database will lose functionality as a trusted identification resource.

Out of the 236 samples with a DNA barcode, 41.2% ($n = 89$) were found mislabeled (Tables I and III). Table III provides an overview of the mislabeling rates detected in different metropolitan areas and venues therein (e.g. fillet purchased from the market versus meal purchased in a restaurant or takeout). Cases of fillet mislabeling do not significantly differ across the various localities examined. However, the combined incidence of restaurant and takeout mislabeling is significantly greater than that of market fillet mislabeling ($p > 0.001$ based on a conditional chi-square test with one degree of freedom). This could be due to a sampling bias, however, because samples collected from sushi restaurants were directed toward species that are known targets of substitution (e.g. red snapper). Non-standardized sample procurement represents an acknowledged shortcoming when performing a retrospective analysis of multiple case studies, yet the pervasive nature of substitution and mislabeling are consistently evident across Canada. Our results are in concert with related studies (e.g. Wong and Hanner 2008; Miller and Mariani 2010), which taken collectively clearly illustrate that seafood mislabeling is widespread and common.

In our study, cod was often substituted (e.g. with *Melanogrammus aeglefinus* or *Gadus chalcogrammus*), as was Red Snapper. The Pacific Salmon samples often lacked required species designation (e.g. Coho, Sockeye, Pink), and were also sometimes substituted with Atlantic Salmon. Halibut was not always correctly labeled as Atlantic versus Pacific. In several cases, Patagonian Toothfish was called Chilean Seabass, a vernacular name that is commonly used despite not being listed as an acceptable market name. Other notable findings include sample CBCMS049-10, labeled as "shark steak" and subsequently identified as *Carcharhinus plumbeus* (Sandbar Shark). Neither the common name nor the species are currently included in the CFIA Fish List. The International Union for the Conservation of Nature classifies it as vulnerable globally, with significant declines estimated and suspected in several areas of its range, (see: http://www.iucnredlist.org/apps/redlist/details/3853/0). However, it should be noted that the so-called *C. plumbeus* reference sequences are scattered across several distinct haplogroups in BOLD.

This highlights several issues. First, only about one-half of the known species of elasmobranchs have been barcoded (Becker et al. 2011), and since there are only a few shark vouchers for those that have been profiled (because of body size constraints with respect to archival), some of these clusters may represent cryptic species, misidentifications, and/or other described species that are not yet clearly identified within the reference database. Most known shark species are well represented and exhibit cohesive barcode clusters that are distinct from those of other known species, but the dataset needs further expansion to include

more expert-identified reference material. While FISH-BOL/BOLD attempts to use such material, it also retrieves data from GenBank, which is not actively curated and is also known to contain inaccuracies (Harris 2003). BOLD does provide a platform to support third-party annotation of suspicious GenBank records and flag them for removal from the BOLD identification engine, but in some cases, there are not enough comparative data to make a clear decision. Second, based on currently unresolved entries in the reference database, there is a slim possibility that the sample might actually be *Carcharhinus altimus* (Bignose Shark). Notably, this species is not included in the CFIA Fish List either. Third, assuming current taxonomic classification to be accurate, the use of additional markers might be necessary to discern certain closely related shark species (e.g. Wong et al. 2009). These issues notwithstanding, for species such as the Sandbar Shark to be sold in Canadian markets, the industry needs to petition the CFIA to include them under an accepted common name in the Fish List. Until this happens, these species should not be entering the human food supply.

Concluding remarks

For consumers, this work illuminates the problem of market substitution and provides an important example of "translational taxonomy" as enabled by barcoding. While a prior forensic study on commercial fish and seafood products in North America uncovered taxonomically widespread mislabeling (Wong and Hanner 2008), this research significantly extends the geographic coverage and depth of sampling over previous investigations, exposing systemic seafood mislabeling across much of Canada. Limited sample sizes and an *ad hoc* sampling schema hinder us from making inferences about relative rates of mislabeling between species, while sampling biases restrict inferences concerning baseline substitution across the entire economic spectrum of available seafood options as a whole. Hence, we cannot explicitly test the hypothesis that inexpensive species are less commonly mislabeled than more expensive species. Yet, we suspect that the substitution of inexpensive species is much less common than the substitution levels observed among the higher priced species exposed in this study. A more balanced experimental design that incorporates samples from across all seafood price categories, regions, species, and major importers would certainly prove informative. A conservative worldwide substitution rate of just 10% (as detected in the fillet samples from this study) would implicate US$S24 billion (10% of US$S240 billion *cf*. Dyck and Sumaila 2010) in fraudulent seafood shipped annually worldwide, a statistic that when combined with substitution levels as documented in this study should provoke more thorough investigations.

While demonstrating the utility of barcoding, we also highlight taxonomic uncertainty pertaining to some poorly resolved and/or potentially cryptic taxa, thereby flagging targets for further taxonomic inquiry. Although most species of commercial interest are already well characterized both traditionally and more recently with barcodes, more work remains for constructing the reference library as evidenced by the six samples that yielded no close match in either BOLD or GenBank. Regulatory lists serve as "Rosetta Stones" for linking common, market, scientific, and vernacular names and, when combined with DNA barcoding, an innovative solution for detecting and controlling market substitution emerges (Yancy et al. 2008; Handy et al. 2011). This approach should also be seen as an important adjunct to popular certification schemes currently under scrutiny (e.g. Jacquet et al. 2010). Moreover, the uptake of barcode-based approaches for species identification could protect both consumers and retailers from market fraud (and any liabilities this might incur) as well as aid implementation of conservation legislation and catchment monitoring on a global scale.

One unintended consequence of improved monitoring empowered by barcoding could be the elimination of Tilapia substitutions commonly seen in the market, resulting in greater pressure on already depleted stocks of some marine species. Consumer education is crucial and in this respect, we highlight the role of responsible journalism in aiding consumers to make informed choices—not only with respect to economic fraud, but also concerning ethical consumption. The need for accurate and transparent labeling exists not only for species, but also extends to country of origin and capture method if consumers are to be fully capable of exerting market pressures in favor of sustainability.

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Supplementary material available online Supplementary Figure S1