Marketplace substitution of Atlantic salmon for Pacific salmon in Washington State detected by DNA barcoding

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A R T I C L E   I N F O

Article history:
Received 7 June 2011
Accepted 25 October 2011

Keywords:
Market substitution
Atlantic and Pacific salmon
Oncorhynchus
Salmo
DNA barcoding
COI gene

A B S T R A C T

Accurate identification of seafood in the marketplace is an issue of international concern, due to high rates of market substitution of cheaper or more widely available species for expensive or high-demand species. Salmon samples from stores and restaurants throughout western Washington, USA were tested using DNA sequencing of a short section of the mitochondrial cytochrome c oxidase I (COI) gene (DNA barcoding) to identify Atlantic salmon substituted for Pacific salmon. Of 99 salmon samples, 11 (11%) were Atlantic salmon sold as Pacific salmon. More than 38% of restaurant samples were mislabeled to species, while only 7% of store samples were mislabeled. Market substitution rates were significantly greater in restaurants compared to stores, and consistently greater in winter compared to spring, although not significantly. The high market substitution rate in restaurants documents a pressing need for more monitoring and enforcement specifically in restaurants. DNA barcoding is a valuable tool for rapid and definitive authentication of salmon in the marketplace, and should be more widely adopted to discourage market substitution.

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1. Introduction

Pacific salmon are composed of six closely related species within the genus Oncorhynchus: chum (Oncorhynchus keta), coho (O. kisutch), chinook (O. tshawytscha), pink (O. gorbuscha), sockeye (O. nerka) and the Japanese cherry (O. masou) while Atlantic salmon, Salmo salar, is less closely related as it is in a separate genus. Of the Pacific salmon, only coho and chinook are farmed, on a limited scale, while globally over 90% of farmed salmon is Atlantic (Food and Agriculture Organization, 2011).

With more than 2.4 million metric tons of global annual production (Knapp, Roheim, & Anderson, 2007), salmon represent an important international commodity. The United States is one of the main producers of wild salmon, with 42% of the global market share. Only 41% of global salmon production is from wild-caught salmon, with the remainder farmed (Knapp et al., 2007). Global wild salmon harvest levels have remained relatively stable over the past few decades, ranging from 0.8 to 1 million metric tons, while the increase in salmon consumption can be accounted for by the rapid expansion of farmed salmon, from negligible levels in the early 1980s to more than 1.5 million metric tons in 2004 (Knapp et al., 2007).

Farming of carnivorous fish species is inefficient due to the reliance on wild-caught fish. While the salmon farming industry has been able to reduce the feed conversion ratio for salmon from 1.5 in 1997 to 1.3 in 2005, the ratio of wild fisheries inputs to farmed salmon outputs or the “fish-in to fish-out” ratio (FI/FO) averages 5.0; in other words, 5.0 kg of wild fish is required to produce the fish oil and fish meal in the feed for every kilogram of salmon produced (Naylor et al., 2009). These wild fish, mostly small pelagic fish, may be threatened by global overharvesting (Naylor et al., 2000). Salmon farms produce nitrogen and phosphorous contamination from feed and fish waste, as more than half of the added N and P are lost from the pens to surrounding waters and sediments (Wu, 1995). Salmon farms can have detrimental impacts on native salmon populations by spreading parasites and diseases (McVicar, 1997). Escape of Atlantic salmon is surprisingly common; up to 2% of all fish escape (Gross, 1998), and large escape events triggered by storms have released up to 300,000 fish at one time (McKinnell & Thomson, 1997). Escaped Atlantic salmon are capable of interbreeding with Pacific salmon, although their offspring have low survival rates, and escaped fish compete directly with native fish for space, food, and access to spawned eggs (Gross, 1998). Market substitution of Atlantic salmon for wild Pacific salmon increases sales of farmed salmon and, therefore, associated environmental impacts.

Public awareness campaigns such as the Living Oceans Society’s ‘Farmed and Dangerous’ campaign and the Marine Stewardship Council (www.msc.org) certification program have prompted many consumers to choose wild Pacific salmon over farmed salmon, motivated by environmental sustainability and health concerns. These programs rely on consumers’ faith in the accuracy of product information such as wild vs. farmed, species name, and country of origin, but recent reports of alarmingly high rates of market substitution in various
international fisheries markets call this faith into question (Jacquet & Pauly, 2008).

Forensic genetic techniques for species identification provide an essential tool for authentication of marketplace seafood, and should be more widely applied to maintain the credibility of certification by discouraging market substitution (Ogden, 2008). While whole fish can usually be identified based on morphology, the final product purchased by consumers can be much more difficult to verify. Various DNA-based methods such as PCR-RFLPs (polymerase chain reaction restriction fragment length polymorphisms) have been developed for screening of specific types of seafood. For example, a RFLP test for salmon species-level identification was able to distinguish most salmon species (Rasmussen & Morrissey, 2009, Rasmussen, Morrissey, & Walsh, 2010). Increasingly, researchers are applying DNA direct sequencing, as the price of this technique has dropped substantially over the last few years and is now more competitive with the cost of RFLP analysis. In-house costs of sequencing are currently less than US$3 per sample. DNA sequencing has the benefit of being definitive and easily comparable across different studies. In addition, analyses require less than a day in a laboratory equipped with a sequencer, and several days to a week when submitted to an external facility.

Several studies have used DNA sequencing of the cytochrome c oxidase I (COI) mitochondrial gene to identify seafood products and investigate broad patterns in the mislabeling of seafood. In a blind study, Yancy et al. (2008) found that sequencing the COI gene definitively identified all tested seafood species, including several salmonids. Wong and Hanner (2008) used this method to show that 25% of seafood samples from the northeastern USA and Canada were potentially mislabeled. DNA sequencing studies focused upon particular fisheries have also exposed consistently high levels of mislabelling; rockfish (Logan, Alter, Haupt, Tomalty, & Palumbi, 2008), tuna from sushi restaurants (Lowenstein, Amato, & Kolokotronis, 2009), and red snapper (Marko et al., 2004) in the USA, cod (Miller & Mariani, 2010), grouper (Asensio, Gonzalez, Pavo, Garcia, & Martin, 2008), and hake (Machado-Schiaffino, Martinez, & Garcia-Vazquez, 2008) in Europe, and various endangered local species in South Africa (von der Heyden, Barendse, Seebregts, & Matthee, 2010) all had rates of market substitution at 25% or higher.

The Food and Drug Administration standard method to detect wild vs. farmed salmon uses high pressure liquid chromatography (HPLC) to measure ratios of astaxanthin to other carotenoid pigments in the salmon flesh (Turujman, Warner, Wei, & Albert, 1997). Using a test of carotenoid pigment ratios similar to that used by the FDA, a New York Times study showed that 6 of 8 salmon samples from restaurants tested as farmed rather than wild, as labeled (Burros, 2005). A similar study carried out by Consumer Reports (Anon. 2006) showed that, during the summer (when wild fish were easily available), 100% of samples tested were accurately labeled, but in November and December, 41% of salmon samples labeled wild tested as farmed. Farmed salmon can also be detected based on lipid ratios sometimes combined with isotopic ratios (Megdal, Craft, & Handelman, 2009; Thomas et al., 2008). In a study of farmed and wild Atlantic salmon from Norway, Ireland, Scotland, Canada, and Tasmania, 9 of 54 or 17% of market samples labeled ‘wild salmon’ were in fact farmed and four of the nine samples were actually trout (Thomas et al., 2008). While COI genes cannot determine whether a salmon was farmed or not, they do provide a definite identification at the species level, and can thus reveal cases of substitution of one species for another. The author is not aware of any other published DNA sequencing-based analyses of market substitution focused specifically on salmon.

In Washington State, wild salmon are a powerful cultural icon. The recent listing of 13 salmon populations within Washington State as endangered or threatened under the Endangered Species Act has raised the visibility of salmon conservation issues. Washington residents may be likely to choose wild salmon over farmed, making this an ideal area to investigate the prevalence of market substitution of salmon. The objective of this study was to measure rates of market substitution of Atlantic salmon for the five species of Pacific salmon commonly sold in Washington State retail outlets.

2. Methods

2.1. Sample collection, PCR, and sequencing

Salmon samples were collected from stores and restaurants in western Washington by students in a college-level introductory biology course, as part of an educational lab experiment developed by the author (Cline & Gogarten, in press). Sampling occurred opportunistically, during winter and spring quarters when the course was taught, from 2009 to 2011. Students requested samples from stores and restaurants to use in an educational exercise, but did not disclose the exact nature of the experiment to avoid potential bias in cooperation with the study. All samples were unambiguously presented as wild Pacific salmon, either in writing (e.g., menu or display case) or verbally by store or restaurant personnel.

An interior section of salmon muscle tissue was excised from each sample, and DNA was extracted using a Mobio UltraClean™ Tissue DNA extraction kit (www.mobio.com). The standard fish DNA barcoding primers, FishF1 (5’ TCAACACCCACAAAGACATTGCGAC 3’) and Fish R1 (5’ TAGACTTCTGGTGCCCGAAATCA 3’) (Ward, Zemlak, Innes, Last, & Hebert, 2005), were obtained from Invitrogen and used to amplify a section of the COI gene. Reactions were performed in 50 μl volumes containing final reaction concentrations of Promega GoTag Flexi DNA polymerase (2.5 U), MgCl₂ (2.5 mM), 1 x buffer, Promega dNTPs (0.2 mM), Fish F1 primer (25 nM), and Fish R1 primer (25 nM). DNA extracts were diluted 1:200 with DNase-free water, and 25 μl of diluted DNA extract was added to 25 μl of the master mix. The PCR reaction conditions were: 96 °C for 5 min, 42 cycles of 94 °C for 60s, 51 °C for 45s, and 72 °C for 60s, followed by a final extension of 72 °C for 5 min. PCR reactions were performed using a ThermoElectron Px2 thermal cycler. Successful PCR products were sequenced at the University of Washington High-Throughput Sequencing facility (http://www.htseq.org) using FishF1 as the sequencing primer.

2.2. Phylogenetic analysis

Samples were initially screened using the BLAST algorithm (Altschul et al., 1997) to identify closest matching sequences in DNA sequence databases accessed via the National Center for Biotechnology Information website (www.ncbi.nlm.nih.gov). Identifications were subsequently confirmed via phylogenetic analysis. Sequences were curated by deleting regions of low quality and rectifying ambiguities, and added to reference sequences identified by BLAST searches. Many reference sequences were provided by the DNA barcode of life project (Ratnasingham & Hebert, 2007). The sequence alignment and the phylogenetic tree were generated using the software package MEGAS (Tamura et al., 2011). The evolutionary history was inferred using the Neighbor-Joining method (Saitou & Nei, 1987). The bootstrap consensus tree was inferred from 500 replicates (Felsenstein, 1985). Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) is shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura, Nei, & Kumar, 2004) and are in the units of the number of base substitutions per site. There were a total of 645 positions in the final dataset.
Fig. 1. A neighbor-joining phylogenetic tree constructed using MEGA5 depicting relationships among reference sequences from Genbank (labeled as “Ref. species name”) as compared to COI sequences generated by this study that were found to be mislabeled (taxa labeled with B# and “Sold as”). Bootstrap values out of 500 iterations are included as node labels for species clades.
2.3. Statistical analyses

Differences between substitution rates in restaurants compared to stores, and in winter compared to spring were tested using the statistical analysis package PASW Statistics 18 cross-tabulation procedure. Chi-square testing was conducted with one degree of freedom and p values less than 0.05 were considered to be significant.

3. Results

In total, 178 samples were analyzed, of which 99 yielded interpretable sequence results. Of these, 42 were obtained from 29 different restaurants, with some sampled over multiple seasons and years. The remaining 57 samples were obtained from 18 different grocery stores, with some sampled over multiple seasons and years.

Phylogenetic analysis of the full dataset produced clear and well-supported separation at the species level, allowing for definitive identification. For simplicity, the phylogenetic tree shown in Fig. 1 includes only the samples found to be mislabeled, in addition to reference sequences for Oncorhynchus and Salmo species. Of the 99 samples with interpretable sequence data, 11 (11%) were labeled as wild Pacific salmon but proved to be Atlantic salmon (Fig. 2a). Market substitution was more common for restaurant samples ($\chi^2 = 11.91$, p = 0.001), with 10 of 42 (24%) compared to only 1 of 57 (2%) for grocery store samples (Fig. 2a). The substitution rate for one species of Pacific salmon substituted for another was also substantially higher for restaurant samples (Fig. 2a). The substitution rate for one species of Pacific salmon substituted for another was also substantially higher for restaurant samples, with 6 of 42 (14%) compared to 3 of 57 (5%) for store samples (Fig. 2b), but the difference was not significant ($\chi^2 = 2.38$, p = 0.123).

Many of the restaurants with mislabeled salmon were sushi restaurants, and most of the substitutions were a cheaper fish substituted for a more expensive species (Table 1), based on wholesale prices reported by Knapp et al. (2007).

The substitution rate was consistently higher in winter, with 8 of 52 (15%) compared to 3 of 53 (6%) in spring for Atlantic salmon, and 6 of 52 (12%) in winter compared to 3 of 53 (6%) in spring for substitution of one Pacific salmon for another (Fig. 2a,b). The seasonal difference was not significant for substitution of Atlantic salmon for Pacific salmon ($\chi^2 = 2.45$, p = 0.118), nor for substitution of one Pacific salmon species for another ($\chi^2 = 1.03$, p = 0.309).

4. Discussion

The rates in this study, at 11% for stores and restaurants combined, are somewhat lower than the reported 17% substitution rate of farmed for wild salmon, detected using HPLC methods (Thomas et al., 2008). This is not surprising, considering that the COI sequence analysis would not detect substitution of farmed salmon for conspecific wild salmon while the HPLC method should detect both types of substitution. The rates of market substitution for Atlantic salmon observed in this study are also lower than the 25% reported by Wong and Hanner (2008) for various seafood products tested using COI sequence analysis. Because their study included only five salmon samples, all sold as either Atlantic salmon or unspecified, their results cannot be directly compared with this study. Other studies of market substitution for various seafood products other than salmon in the United States have also revealed relatively high rates of market substitution (e.g., Logan et al., 2008; Lowenstein et al., 2009; Marko et al., 2004).

More research is needed to explore substitution rates specifically in restaurants, as many other studies may have included restaurant samples but did not differentiate between restaurants and stores when reporting their results (e.g., Thomas et al., 2008; Wong & Hanner, 2008). The incentive to mislabel may be greater in restaurants than in grocery stores due to the greater price difference; alternately this could be attributed to greater standardization or oversight in stores, especially larger chain stores. In this study, 81% of grocery samples were from large chains while only 33% of restaurant samples were from chains. The strong trend towards higher substitution rates in winter than in spring was similar to the pattern observed by Anon (2006), suggesting that substitution may be driven by scarcity of fresh wild salmon during seasons without salmon runs.

It is important to note that mislabeling could occur at various points in the supply chain, and could represent both accidental and deliberate substitutions. Accidental misidentifications would be expected for the samples used in this study.
primarily within the genus *Oncorhynchus*, but it is also possible that Atlantic salmon escaped from pens could be misidentified as Pacific salmon on the boat or on the dock when caught in wild runs. Nevertheless, the higher rate of substitution for Atlantic salmon than for Pacific salmon in this study argues strongly against accidental misidentifications as the main driver of substitutions; likewise, the higher rate of substitution in restaurants than stores argues that this difference arises at the retail level rather than at the supply end of the chain. This has important management implications as it suggests that stronger monitoring and enforcement are needed at the retail level, in particular for restaurants. Because Washington residents may be more sensitized to the issue of farmed vs. wild salmon, it is possible that the rates of market substitution are lower in Washington State. Additional sampling over a broader geographic area would be needed to confirm whether the substitution rates observed in this study are representative of the United States as a whole. Nevertheless, if this rate were considered representative of the United States as a whole, it would be an interesting exercise to estimate the potential annual financial losses to US consumers due to market substitution of Atlantic for Pacific salmon. In 2004, US consumption of fresh or frozen coho, sockeye, and chinook salmon was reported to be approximately 20,100 metric tons (Knapp et al., 2007). If the 11% substitution rate in this study was considered representative for the United States as a whole, this would represent 2200 metric tons that were instead Atlantic salmon. Precise consumer losses are difficult to estimate due to the difficulty of determining the average market price for salmon in grocery stores and restaurants. For example, a typical seafood restaurant in the US might charge $34/lb ($17 for an 8 oz filet) for chinook salmon while grocery prices for fresh Atlantic salmon might range from $3 to $4/lb (Knapp et al., 2007). Wholesale prices in 2004 were $0.50/lb for fresh Atlantic salmon, $0.36/lb for pink, $0.54 for chum, $1.73 for coho, $1.94 for sockeye, and $4.37 for chinook salmon (Knapp et al., 2007). Assuming an average wholesale price of $2/lb for coho, sockeye, and chinook, market substitution might have cost consumers nearly US$7 million per year in the US alone (2200 metric tons = 4,850,000 lb * ($2 − $0.5 − $1.50) = $7,300,000). Using market prices in this estimate might increase the estimated cost to consumers of fraudulent market substitution. A more accurate estimate of the financial impact of salmon market substitution would require a larger sample size over a wider geographic area, and more detailed information about annual salmon purchases, but this rough estimate does serve to illustrate the potential impact for consumers. Jacquet and Pauly (2008) performed a similar calculation for international salmon markets based on a 25% substitution rate, but their estimate of US$2 million cost to consumers globally is likely an underestimate of the true financial losses to US consumers. Based on the results of this study, there is a need for increased inspection of retailers, particularly restaurants; this combined with substantial penalties might greatly reduce the incidence of market substitution.

**Acknowledgments**

I would like to thank my co-instructor Jennifer Gogarten, and the students of TESC130 Introductory Biology II, Cell and Molecular Biology for their diligence and enthusiasm in carrying out this project. Thanks also to Teresa Turk for her helpful comments on the manuscript.

**References**


