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Community engagement in seafood identification using DNA barcoding reveals market substitution in Canadian seafood

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Abstract: Seafood authenticity is a global concern. As seafood consumption increases, so does public awareness of the associated nutritional and environmental issues related to seafood mislabeling. Cases of substitution continue to be observed, even after the adoption of DNA barcoding as a regulatory tool by the Food and Drug Administration in the United States in 2011. Although media coverage of these cases has highlighted the incidence of fraud in Canada, more in-depth engagement of the public is lacking. By partnering with community members to conduct research, knowledge about the incidence and impact of seafood mislabeling can be directly communicated to consumers. In this study high school students and educators participated in a market survey using DNA barcoding to identify seafood. The Canadian Food Inspection Agency Fish List was used to determine if mislabeling had occurred. Twenty-three percent of samples surveyed were mislabeled, suggesting that the incidence of retail seafood mislabeling continues to be significant in Canada. Continued involvement of the public in market surveys will help to monitor trends in seafood mislabeling, and may help to increase awareness of potential seafood fraud.

Keywords: DNA barcoding, seafood identification, market survey, food fraud, citizen science

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1 Introduction

Food fraud is an issue of socioeconomic concern globally. Intentional substitution or mislabeling of species is one form of fraud that has obvious economic implications to consumers when a lower-cost product is labeled as one with a higher value. Price differences between the species of fish on the label and the one actually present in the product can be up to 244% [1]. There can also be health implications as different species have varying levels of heavy metals [2] and nutritional value [3] or may even be toxic [4]. Consumers may also make choices to purchase sustainable species of seafood, however at times "at-risk" species can be marketed as sustainable alternatives [eg. 5,6]. In addition to the impacts to sustainability of fisheries, the economic losses from illegal and unreported fishing have been estimated at between $10 and $23.5 billion annually [7].

Seafood consumption is also on the rise [8]. With globalization of trade it can be difficult to track and authenticate seafood products. This creates the possibility for both intentional and unintentional misrepresentation of products. To combat this, regulatory bodies must utilize new methods for authenticity testing. DNA barcoding is a method that takes advantage of differences in the DNA sequence of a standard gene region in order to identify species [9]. By genetically profiling expert-identified reference specimens [10] the resulting "look-up table" can be used to identify an unknown sample by its barcode (eg. using the Barcode of Life Data System or BOLD[11]). This is particularly useful when diagnostic morphological characters are removed, for example during seafood processing. While errors in identification can be common in some public databases like GenBank [12] where little information aside from the nucleotide sequence is included with results, BOLD was developed specifically to serve as a DNA barcode library where additional information regarding sample provenance and raw sequence files can be uploaded with samples [11]. Many entries have voucher

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specimens or tissue that can be re-examined, and this publically available, well-populated reference database is a useful tool for food authentication [13].

DNA barcoding has been gaining in popularity as a rapid and accurate method for species identification of seafood products. In 2011 it was adopted by the United States Food and Drug Administration (FDA) as the primary method of regulatory control of seafood products in the United States [14]. Moreover, DNA barcoding has been used in several seafood market surveys [5,6,15-20]. These studies have continued to shed light on instances of fraud found in the seafood industry around the world. Additionally, the media coverage of instances of food fraud has made this information available to citizens [21].

Here we take this one step further by encouraging citizen involvement in a consumer-driven market survey conducted in Canada. Together with Let's Talk Science, a Canadian not-for-profit organization dedicated to science outreach, a seafood market survey and related educational outreach was conducted between September 2012 and April 2013. High school teachers were provided with tools to incorporate DNA barcoding concepts into the classroom curriculum and the method was subsequently used to identify seafood products gathered by high school students from their local grocery stores.

2 Methods

2.1 Sample Collection

Teachers within the existing network of educators in Ontario (and one school in Manitoba) were contacted and able to sign up their class to take part in the survey. To increase interest, supplementary lessons covering aspects of the seafood supply chain and DNA barcoding were also provided online (http://www.explorecuriocity.org/Community/ActionProjects/MarketSurvey.aspx) and could be accessed after free registration as an educator or student. This allowed students and teachers to access different portions of the materials and restricted access to assignment answers. Participating educators were invited to attend a workshop at the Biodiversity Institute of Ontario to review DNA barcoding concepts and lesson plans. Instructions on sample collection were given in the form of a lesson plan that could be shared with students and included a list of suggested products to sample in an effort to limit the types of seafood purchased in order to streamline collection and analysis. The following market names were suggested: salmon, bass, snapper, tilapia, basa, shark, halibut, haddock, cod, catfish, pickerel, whitefish, perch, orange roughy, sole and pollock. Any samples received that were not labeled with one of the suggested market names were grouped together in a single category labeled "Other" for analysis of the frequency of mislabeling in certain products. Samples were requested to be fresh or fresh frozen only.

After creating a sampling plan in class to minimize overlap in the species collected and stores visited, students went to a local grocery store or market to purchase their seafood products. Data collection sheets were filled out for each sample by the students, including information such as price, location of collection and photograph of product package. We provided each teacher with 1.5mL microcentrifuge tubes, each with a unique sample ID number and tracking barcode. Small (~ 2 cm^3) tissue subsamples of each product were collected by students, and deposited into the provided tube and preserved by immersion in 95% ethanol.

2.2 DNA Barcoding

Samples were analyzed at the Canadian Centre for DNA Barcoding or at the Centre for Biodiversity Genomics at the University of Guelph using standard DNA barcoding protocols and primers for amplification of the DNA barcode region of COI as outlined by Wong and Hanner [5]. Failures were amplified and sequenced again using AquaF2/C_FishR1T1 primers as detailed by Ivanova et al. [22].

2.3 Species Identification

The DNA barcode sequences recovered from the submitted specimens were queried against the BOLD identification engine's "species level" search to determine if an unambiguous species-level match could be made with a sequence similarity of 98% or higher. If the sequence could not be identified using BOLD, an NCBI BLAST search of GenBank was used.

The species name determined from the DNA barcode was then compared to the corresponding market name(s) included in the Canadian Food Inspection Agency (CFIA) Fish List for that species. If the market name from the sample package was listed under the species name obtained from BOLD on the CFIA Fish List, the sample was considered correctly labeled. If not, or if the species name was not found on the CFIA Fish List, the sample was considered mislabeled.

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3 Results and Discussion

Compliance with specimen data collection requirements was excellent. Just sixteen samples of 310 submitted (all from one school) failed to meet our inclusion criteria. This high level of compliance was likely due to the training workshop held for teachers and the incorporation of online resources for teachers and students participating in the project [23]. DNA barcoding is a useful way to illustrate some of the basic molecular biology techniques included in high school curriculum and allows students to address applied scientific questions using discovery based learning and real-world examples [23,24]. The classroom resources developed in collaboration with Lets Talk Science for this project can be used to facilitate the introduction of DNA barcoding as an example of molecular biology in action. They will also help teachers and students contribute more meaningfully to citizen science projects related to DNA barcoding in the future by providing guidelines for formalizing and standardizing collection and analysis of samples.

In this market survey, DNA barcodes were obtained from 294 samples (Supplementary Table 1). All but one barcode sequence had a match in BOLD of at least 98%. This sample could not be identified using BOLD or GenBank. In total, 67 of the remaining 293 products with DNA barcodes (23%) were identified as mislabeled using the CFIA Fish List as a guide. Figure 1 shows the incidences of mislabeling according to market name. This level of mislabeling is similar to that found in previous market surveys [5,6,15-20,25]. High levels of mislabeling, defined as over 50% of collected samples, were found in shark (8/8), red snapper (7/9), whitefish (3/4), snapper (5/8) and bass (8/15) samples. We found between 25%

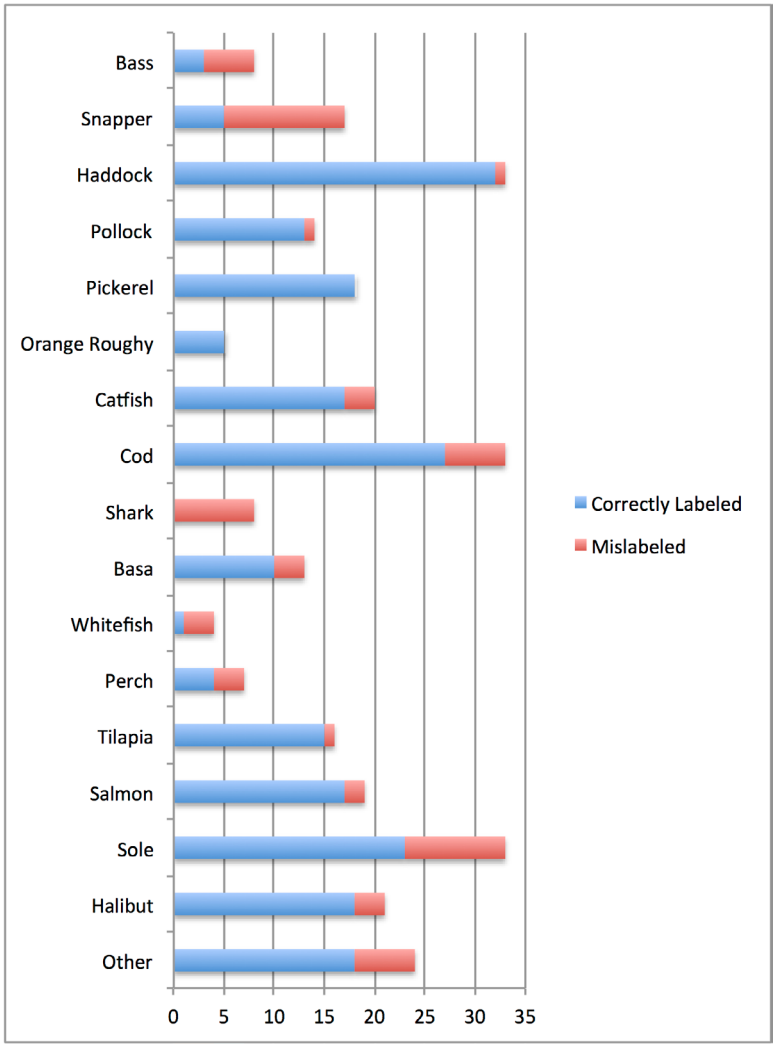

Figure 1. Summary of incidences of sample mislabeling according to market names.

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and 50% mislabeling in sole and perch as well as in those samples listed in the "other" category. Samples with the following market names were received and included in the "other" category: tuna, swordfish, rockfish, mussel, yellowtail, walleye, flying fish roe, artificial crab, Alaskan snow crab, eel, rainbow trout, and monkfish. No specific species seem to be consistently substituted for any of these market names (Supplementary Table 1). For example *Rhomboplites aurorubens*, *Lutjanis synagris*, *Oreochromis* sp. and *Sebastes viviparus* were all found substituted for red snapper.

Of the 67 cases classified as mislabeled, 30 were straightforward examples where the species determined with DNA barcoding was found on the CFIA Fish List and did not match the market name listed on the product. For example, tilapia (*Oreochromis* sp.) was substituted for red snapper and cod. Tilapia is cheaper than both cod and red snapper, and substitution might be considered a case of economic fraud. Farmed tilapia also have a higher incidence of elevated environmental contaminants, such as heavy metals and carcinogens, than many wild caught species [26], illustrating potential impacts of mislabeling on human health and emphasizing the link between species authentication and food safety. Previous studies have also focused on the impacts of incorrect labeling of snapper species on conservation and consumer choice as these species are often slow to reproduce and may be overfished [27], demonstrating the potential environmental impact of mislabeling. Another sample in this study showing a possible environmental impact of mislabeling was marketed as bass but was identified as *Dissostichus mawsoni*, Antarctic toothfish. Although this species has not yet been assessed by the International Union for the Conservation of Nature (IUCN), there has been concern about the effect of industrial fishing on the Antarctic toothfish in the Ross Sea on not only populations of *D. mawsoni* [28], but also other species that prey on them [29].

The species determined with DNA barcoding for the remaining 37 samples suspected of being mislabeled were not included on the CFIA fish list. These were considered mislabeled according to the CFIA regulations for food labeling in Canada. However, in order to determine the relationship between the species identified using DNA barcoding and the market label, FishBase was used to establish the common name of these 37 samples to compare to the market labels. In 10 of these cases, identification of common name using FishBase showed that the common name of the sample did not match the market name. These 10 cases were therefore similar to the straightforward examples of mislabeling in the category described above. For example one sample was labeled halibut, but was determined to be *Hypothordus flavolimbatus*, or yellowedge grouper, listed as vulnerable by the IUCN.

The other 27 were cases where the common name of the species as determined by FishBase did match the market name. Although in these cases the common names did match the market labels, these samples were still considered mislabeled due to the absence of the corresponding species from the CFIA Fish List. All eight of the shark samples fall into this category. Although none of the three species, identified from the shark samples using DNA barcoding (*Carcharhinus brevipinna*, *Carcharhinus limbatus, and Carcharhinus tilstoni*) were listed on the CFIA Fish List, they are all species of shark. Although on the surface this may seem legitimate for samples labeled as shark meat, *C. brevipinna* and *C. limbatus* are listed as near threatened by the IUCN, illustrating possible conservation implications of non-compliant seafood labels.

All but one mislabeled sole sample also fell into this category. The samples were identified as *Lepidopsetta polyxsystera and Solea solea*; according to FishBase northern rock sole and common sole respectively. Interestingly, *Solea solea* appears on the FDA Seafood List as one of the acceptable species for the market name sole, but not on the CFIA Fish List. This illustrates the vagaries that exist between jurisdictions and the complexities of labeling products in a global market. These examples also highlight the need for an accepted communication framework governing the trade of seafood internationally that is regularly updated. If a harmonized list of species and corresponding market names existed, it may reduce some of the difficulties in assessing rates of mislabeling and aid in separating economically motivated substitution from miscommunications related to regional nomenclature or out-of-date lists.

Instances of mislabeling and differences between regional fish lists underscore the benefits of labeling seafood products with their scientific names (e.g. Latin binomial nomenclature consisting of genus and species) in regions where this is not already part of seafood legislation, which has been advocated recently [e.g. 18]. Species labeling would not only aid in the detection of market substitution, but could impact consumer choice on products that are not mislabeled. For example, there were 31 samples collected in this study labeled only as "cod". Of the 25 that were not mislabeled, 7 were Atlantic Cod (*Gadus morhua*), which is considered a less sustainable fish option than the 18 Pacific cod (*Gadus macrocephalus)* samples; this trend that has been identified in previous

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market surveys [30]. Differentiating these two species on the label could assist consumers in making a more informed choice on their seafood consumption.

4 Conclusion

DNA barcoding continues to be a useful tool in the detection of market substitution in seafood. Overall, 23% mislabeling was observed in this study and revealed instances of substitution with potential economic, conservation and health impacts. Increased consumer awareness of these practices, in combination with accurate means of identifying mislabeling, such as DNA barcoding, may help discourage future seafood mislabeling. Indeed, economically motivated circumvention of labeling regulations have recently been identified and successfully prosecuted [31]. Citizen science projects can improve public familiarity with complex issues, and also expose individuals to the scientific method in a meaningful real-world context, allowing them to use contemporary tools to contribute to larger research questions of socioeconomic importance. We encourage continued involvement of communities in similar studies, particularly in partnership with the scientific community. Although data generated from citizen science projects is unlikely to adhere to the stringent workflow required for regulatory purposes, the increased community awareness and media coverage of seafood mislabeling may help drive improvements in regulation and labeling, as suggested by a recent study [21]. In this way students can contribute meaningfully to addressing issues of global importance by applying the scientific principles learned in the classroom.

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Conflict of interest: Dr Naaum has nothing to disclose.

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