Tuna Labeling practices on the campus of the University of British Columbia

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Abstract

Seafood fraud has been a longstanding issue among Canadian markets where species of fish are often mislabelled and sold to consumers under pretenses. Oceana.ca is a non-profit organization that has been at the forefront of uncovering the truth behind seafood mislabelling, running several thorough studies across Canada to determine which foods are most affected. Given the severity of seafood mislabelling in the region, this project was designed to determine whether the problem at hand is occurring locally at the University of British Columbia, particularly with foods labelled to contain tuna. This project divided several food establishments owned and run by two different operators to deduce whether a difference would be noted in the quality and integrity of food that students across campus have access to. Two establishments were chosen per operator, and three samples were collected from each establishment. For this research, the operators are referred to as Operator A and Operator B. To acquire the results, DNA isolation, PCR, and gel electrophoresis were performed on each sample. Initial testing using tuna-specific primers showed that only 1 establishment from Operator B displayed band separation in the gel, indicating that only this sample contained tuna. Because of this, the samples were run once more using salmon primers as a control. This resulted in banding, thus confirming the appropriate quality of fish DNA. NCBI's BLAST databases were used to compare the genomic sequencing of the samples, resulting in matches for *Lepidocybium flavobrunneum* (Escolar) and *Katsuwonus pelamis* (Skipjack). Future research is necessary to determine whether this is a consistent result. It is suggested more samples be collected over a longer period.

Introduction

Seafood fraud refers to the mislabelling, misrepresentation, or substitution of seafood products, often to deceive consumers with cheaper alternatives for a distributor's financial gain. Not only are cheaper species substituted, but oftentimes false information on the origin, weight, and quality of the seafood is also provided. Oceana Canada has run several tests throughout the years to narrow down the percentage of food affected in the country. A study from 2010-2012 collected more than 1,200 samples of seafood and found snapper and tuna to have the highest rates of mislabelling, tuna resulting in 59% of samples being mislabelled (Warner et. al, 2013). The study was run again in 2017-2018 using 382 samples, and yet again found that 41% of the tuna tested was not tuna, but the fish Escolar (Levin, 2018). Oceana Canada was determined to continue researching seafood fraud and in 2021 found the problem to be consistent, with an

overall mislabelling rate across all seafood of 47%. Tuna was once more found to be Escolar. Oceana Canada reports that even with government knowledge, the problem is persistent and more efforts are necessary to reduce seafood mislabelling long-term (Thurston, 2021).

With the increasing concerns, the Government of Canada has attempted to remediate the issue by implementing DNA testing, and more strictly enforcing fishing and food production policies. In a press release by the Canadian Food Inspection Agency, the public was given confidence in the future of seafood handling and labelling (CFIA, 2021). The agency noted:

> *"Under the Food Policy for Canada, launched in June 2019, the Government of Canada invested $24.4 million over 5 years to the CFIA to tackle food fraud to protect consumers from deception and companies from unfair market competition. With this funding, the CFIA is conducting inspections, collecting samples, testing foods for authenticity, and gathering intelligence to better target its oversight activities."*

Researchers at Dalhousie University in Nova Scotia, however, note that it is exceedingly difficult to trace a fish from ocean to plate and highlight that many food suppliers may also be falling victim to mislabelling, rather than being the cause (Foran, 2021). Although a complex problem, as research advances in the fields of science, fishing, fisheries, law and policy-making, improvements for consumers, suppliers, and producers may be attainable shortly.

Methods

Sample collection: 2 vendors were chosen from both Operator A and Operator B to collect tuna. To increase the sampling variety, 3 samples of tuna were obtained over a few days and stored in a refrigerator from each of the four vendors, for a total of 12 samples.

DNA isolation

A small piece of tuna from each of the initial samples was added into sterile 1.5mL Eppendorf tubes and was mashed with a toothpick. Then, 300 µl of Cell Lysis Solution with Proteinase K was added to the tubes and was left to incubate at 65℃ for 15 minutes with vortexing occurring every 5 minutes until the solution became cloudy. Following incubation, the samples were placed on ice for 5 minutes before adding 150 µl of Protein Precipitate Reagent into the tube, and then vortexing again for 10 seconds. The samples were then centrifuged for 10 minutes at maximum speed, making sure that the tubes were balanced within the centrifuge. After 10 minutes was completed, the supernatant was carefully transferred to a new tube, ensuring that the pellet was not disturbed. Then, 150 µl of ice-cold isopropanol was put into the new tubes containing the supernatant and the tubes were inverted 30-40 times. The tubes were placed back into the centrifuge at maximum speed for 10 minutes before pouring off the isopropanol, leaving a very DNA small pellet. 500 µl of ethanol was added to the pellet for

rinsing and was poured off. The tubes were left lying on their side with the cap open overnight so the remaining ethanol could evaporate.

PCR

30 µl of TE buffer was added to each of the dry DNA pellets and mixed with a pipette to dissolve before the Master Mix was created. The values of each of the components of the Master Mix were multiplied by 15 so the final Master Mix was enough to fill 15 PCR tubes to account for the 12 samples, 1 control, and 2 "ghosts" in case there were any errors along the way. Over ice, 75 µl of 50% glycerol, 37 µl of 10X PCR buffer, 22.5 µl of 25 mM $MgCl_2$, 22.5 µl of Ala-F Primer 10 µM, 22.5 µl of Ala-R Primer 10µM, 21 µl of Obe-F Primer 25 µM, 21 µl of Obe-R Primer 25 µM, 21 µl of dH_2O, 18 µl of Kat-F Primer 25µM, 18 µl of Kat-R Primer 25 µM, 18 µl of Alba-F Primer 25µM, 18 µl of Alba-R Primer 25 µM, 15 µl of Thy-F Primer 10 µM, 15 µl of Thy-R Primer 10 µM, 7.5 µl of 10 mM dNTPs, and 7.5 µl of Taq polymerase were added into the Master Mix tube. 24 µl of the made Master Mix was then added into 13 PCR tubes to be placed in the PCR machine. 1 µl of each of the dissolved DNA was then added into the 12 PCR tubes, and 1 µl of dH_2O was added into the last PCR tube to act as a control. The 13 tubes were placed into the PCR machine and ran at a cycle of 95℃ for 5 minutes, 95℃ for 30 seconds x40, 62℃ for 30 seconds x40, 72℃ for 30 seconds x40, 72℃ for 5 minutes, 4℃ overnight, and then was stored in a freezer.

PCR was also carried out on the samples with the use of salmon primers to detect if salmon was present in any of the samples.

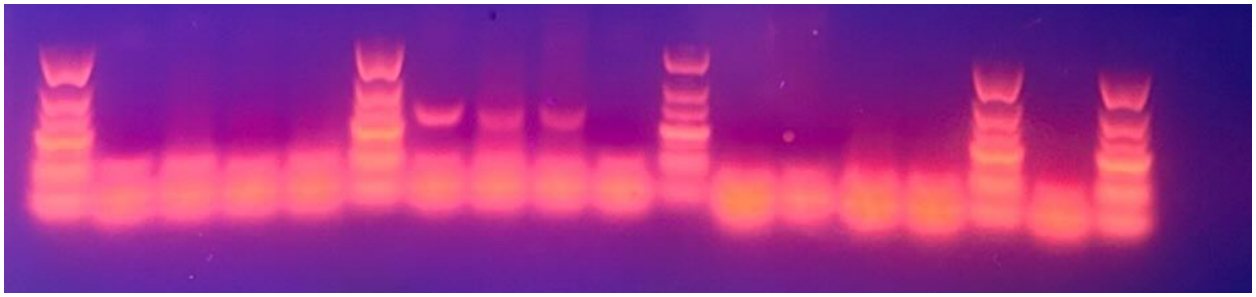
Electrophoresis

5 µl of each of the PCR tuna samples was pipetted onto a piece of parafilm, with 1 µl of 6X loading dye being placed into all of the PCR tuna samples as well. The entire drop is loaded onto the 3% gel, and the same is repeated for the 13 samples with a fresh tip being used in between each drop. The ladder was also loaded into the gel. The samples initially ran at 50V for 10 minutes, before rising to 150V for 45 minutes. The samples that used salmon primers were run on a 1% gel run at 120V for 1 hour.

The results of each of the 12 samples were then compared with each other, as well as with the banding pattern for the various tuna species from literature.

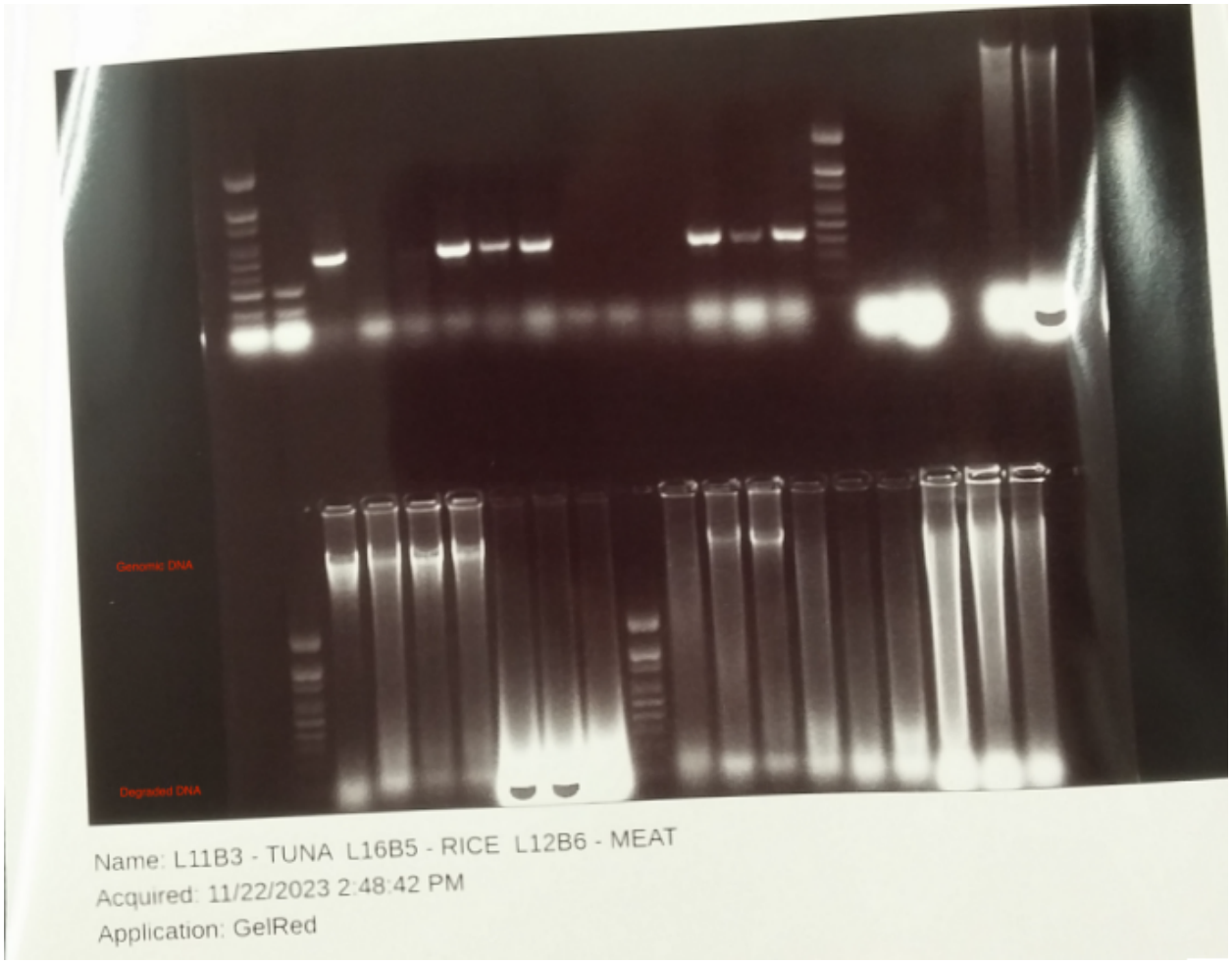
Results

Figure 1. Tuna Primer, Gel Bands



This figure shows the DNA bands of Tuna From the venues noted by B,C,D, and H. Each column represents the following respectively: Ladder, Control, C1, C2, C3, Ladder, B1, B2, B3, D1, Ladder, D2, D3, H1, H2, Lader, H3, Ladder

Figure 2. Salmon Primer, Gel Bands



In the above figure, the top row of the gel portrays the DNA bands that appear using ‘salmon primers’ from the venues noted by B,C,D, and H when run using PCR and gel electrophoresis techniques.

Figure 3. Basic Local Alignment Search Tool (BLAST) Search Results

1. *Lepidocybium flavobrunneum* (Escolar): F primer 100% match; R primer: 1 nucleotide
    a. GCI**C**CTCARAATGAYATTTGTCCTCA
    b. GCI**A**CTCARAATGAYATTTGTCCTCA
2. *Thunnus obesus* (Bigeye): F 1 nucleotide difference; R primer no result for Blast
    a. AAAAACCACCGTTGT**T**ATTCAACT
    b. AAAAACCACCGTTGT**A**ATTCAACT
3. *Katsuwonus pelamis* (Skipjack): F primer 1 nucleotide difference; R primer (100%)
    a. AAAAACCACCGTTGT**T**ATTCAACT
    b. AAAAACCACCGTTGT**A**ATTCAACT
4. *Thunnus thynnus* (Atlantic bluefin): F 1 nucleotide difference; R primer no result for Blast
    a. AAAAACCACCGTTGT**T**ATTCAACT
    b. AAAAACCACCGTTGT**A**ATTCAACT
5. *Thunnus alalunga* (Albacore): F primer 1 nucleotide difference R primer: missing 1 nucleotide
    a. AAAAACCACCGTTGT**T**ATTCAACT
    b. AAAAACCACCGTTGT**A**ATTCAACT
    c. GCICCTCARAATGAYATTTGTCCTCA
    d. _CICCTCARAATGAYATTTGTCCTCA
6. *Thunnus albacares* (Yellow fin): F 1 nucleotide difference; R primer no result for Blast
    a. AAAAACCACCGTTGT**T**ATTCAACT
    b. AAAAACCACCGTTGT**A**ATTCAACT

This figure shows the results of the BLAST query run by the Lab Technician, Mindy Chow. BLAST compares unknown DNA sequences to previously identified samples, searching for similarities in the protein or nucleotide sequence. 100% matches were found for *Lepidocybium flavobrunneum* (Escolar) for the F primer, one nucleotide difference for R primer; *Katsuwonus pelamis* (Skipjack) for the R primer, one nucleotide difference for F primer.

Discussion

This discussion focuses on the analysis of the DNA gel bands obtained in this study, taking into consideration both the presence and absence of these bands and the process in which was taken to establish them across the 12 different samples.

As can be seen in Figure 1, banding patterns are only present in the labelled B Tuna samples, with each band at the approximate value of 150bp. This consistency across the samples indicates that the Tuna are sourced the same.

Conversely, the three other venues, two of which belong to Operator A, showcase an absence of any bands in the gel from Figure one. This absence may indicate that the samples from these venues do not belong to any of the tuna species examined in this study unless experimental errors occurred during the process. To determine the identity of these samples, additional experimentation was necessary. The samples were run using salmon primers as a control to determine whether the quality of the DNA isolated was appropriate. This new experiment showed banding patterns for most all samples, indicating that there was appropriate fish DNA. Given that the salmon primers work well for many species, the banding patterns simply indicated that it is fish but the species would need to be narrowed down further. A BLAST search was run using the genomic data from the alleged tuna samples. This query showed the most similarities with *Lepidocybium flavobrunneum* (Escolar) and *Katsuwonus pelamis* (Skipjack).

Provided that banding patterns developed with one vendor during the initial tuna primer experiment, and Skipjack was a match under BLAST sequence comparisons, it can be said that the initial results were for the Skipjack species of fish. Concerningly, however, Escolar was also a match among the sampled sequences. This species of fish, although legal for sale and use in Canada, is heavily looked down upon for its potential side effects upon consumption such as diarrhea, vomiting, and nausea. Escolar has been previously banned in Italy, South Korea, and Japan (CIFS, 2018).

Given that the second round of gel electrophoresis and the BLAST query were conducted separately by the lab technician, full interpretations and absolute conclusions cannot be guaranteed as there are elements of the methodology that the primary researchers are not well versed in.

It is also significant to note that this experiment utilized a total of 12 samples sourced from four different vendors. One vendor provided canned tuna rather than raw as the others did. This could potentially introduce metal contamination that might obscure experimental results for this vendor. The smaller sample number of this study is likely a limitation that played a factor in the results of the study.

Other causes for errors in this study may have occurred, such as contamination of the samples during collection or handling, issues with DNA extraction, and issues with PCR amplification. To help negate this, more caution could be taken when considering which vendors from Operator A and Operator B to take samples from, such that they are all in the same raw

state. As well as taking a larger sample size, experimenting on them in ‘batches’ such that any error would not be uniform and thus be easier to detect.

Conclusion

From the first examination of 12 samples, it was observed that Operator A had a 50% mislabeling rate, while the mislabeling rate at Operator B reached 100%, resulting in an average mislabeling rate of 75% between them both, as so far can be concluded with the results gathered in this experiment. The existence of bands when using salmon primers confirms the presence of real fish in the sushi of all vendors, this fish including Escolar and Skipjack species as confirmed by the BLAST search.

Notably, Venue B demonstrated accurate labelling for tuna in all three samples tested at their establishment, which was concluded to likely be Yellowfin tuna**.** However, for Operator A, the lack of results for certain species raises uncertainty about the accuracy of tuna labelling in their samples, as such it has been concluded that they are entirely mislabeled.

Further investigation is warranted, particularly focusing on AMS, to explore the possibility of samples being of Tuna species unaccounted for in this experiment. This calls for additional testing involving the selected vendors in this study. With the increase in samples taken from an increased number of vendors for both Operators, more information can be added to this study to create a higher confidence in the true labelling practices on campus at the University of British Columbia.

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Appendix

Tuna PCR Protocol and Master Mix Calculation

Reagents	Amount per tube	Amount per 15 tubes
10X PCR buffer	2.5 µL	37.5 µL
10 mM dNTPs	0.5 µL	7.5 µL
25 mM $MgCl_2$	1.5 µL	22.5 µL
Obe-F Primer 25 µM	1.4 µL	21 µL
Obe-R Primer 25 µM	1.4 µL	21 µL
Kat-F Primer 25 µM	1.2 µL	18 µL
Kat-R Primer 25 µM	1.2 µL	18 µL
Thy-F Primer 10 µM	1.0 µL	15 µL
Thy-R Primer 10 µM	1.0 µL	15 µL
Ala-F Primer 10 µM	1.5 µL	22.5 µL
Ala-R Primer 10 µM	1.5 µL	22.5 µL
Alba-F Primer 10 µM	1.2 µL	18 µL
Alba-R Primer 10 µM	1.2 µL	18 µL
Taq Polymerase (1000U/2000µL)	0.5 µL	7.5 µL
50% Glycerol	5.0 µL	75 µL
dH_2O	1.4 µL	21 µL
Total	24 µL	120 µL
DNA	1.0 uL	

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