"Evaluating Proper Labeling of Raw Yellowfin and Albacore Tuna Through Species Identification Analysis"

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Abstract

This observational study aims to investigate the accuracy of labelling in raw tuna by testing DNA samples from two species sourced from 8 locations. The two tuna species used are albacore and yellowfin, and each sample was obtained from different grocery stores and sushi restaurants. The techniques involved are DNA isolation, PCR, and gel electrophoresis to identify potential mislabelling of tuna species. In DNA isolation, samples were treated with cell lysis solution and Proteinase K, followed by precipitation with isopropanol and ethanol to obtain DNA pellets. PCR was performed using a master mix containing primers specific to tuna and Taq Polymerase, allowing for DNA amplification of 40 cycles. Gel electrophoresis was conducted to compare band patterns and detect DNA markers specific to tuna. The literature values of band patterns for yellowfin and albacore were 127 bp and 178 bp, respectively. Based on data from the gel electrophoresis, we obtained only 4 results from the 8 samples we had since 4 of them returned inconclusive banding patterns. Three of the four banding pattern results were for Yellowfin samples and one was for an Albacore sample. The three Yellowfin samples were labeled correctly while the Albacore sample was mislabeled.

Introduction

Seafood mislabelling is a global phenomenon, with over 30 countries having an average of 36% mislabelled seafood. Canada, being one of those countries, has a 55% mislabelling rate (Watts, 2021). This is concerning as consuming mislabelled seafood can lead to many health problems. Such problems include ingestion of neurotoxins such as mercury which is a high threat to children and pregnant women, as well as other allergies and substances (Silva et al., 2021). In 2007, monkfish (*Lophius piscatorius*) was sold in the United States which caused 2 people to fall ill after consumption. Later, it was found that the monkfish was mislabelled, and had turned out to be pufferfish which contains a potentially lethal neurotoxin called tetrodotoxin (Cohen et al., 2009). In addition to health concerns, seafood mislabelling also has negative impacts, undermining conservation efforts. This type of overfishing can lead to consequences such as threatening endangered species (Logan et al., 2008).

While there exists many studies of seafood mislabelling in places across the world, we are more interested in a locally studied mislabelling rate. Our tuna, which will be obtained in British Columbia and the focus of this paper, is also included in the category of mislabelled seafood. Another study found that 43% of their albacore tuna (*Thunnus alalunga***)** was mislabelled (Sotelo et al., 2018). Therefore, we suspect that at least 6 of our 8 samples will be mislabelled. This statement accounts for all 4 of our yellowfin samples, and 2 of our 4 albacore samples. Our results will be obtained through DNA isolation and polymerase chain reaction (PCR), and will be examined through gel electrophoresis.

Methods

DNA ISOLATION

Initially, gloves were put on to lower the chance of contamination with your own DNA. After the 8 samples of raw tuna (Albacore and Yellowfin species but from different locations - listed in the results table) were obtained, the tip of each Eppendorf tube was filled with the samples using sterile techniques. Afterwards each sample was smashed with a toothpick. Then, in each tube, 300 uL of Cell Lysis Solution with 1 ul Proteinase K (50ug/ul) was added. After 15 minutes of incubation of tubes at 65ºC, tubes were vortexed every 5 minutes. Afterwards, samples were placed on ice for 5 minutes. 150 ul of protein precipitate reagent was added, then vortexed for 10 seconds. After 10 minutes of centrifuging, sample liquids were transferred to fresh tubes. Fresh tubes were filled with 500 ul of ice-cold isopropanol, then carefully inverted 40 times. After 10 minutes of centrifuging at maximum speed, the isopropanol was carefully removed without disturbing the DNA pellet at the bottom. 500 ul of 70% ethanol was added and

carefully poured off so it didn't disturb the DNA pellets. 500 ul of ethanol was added again and poured off. Tubes were left overnight on their sides at room temperature with the caps open.

PCR

30 ul of TE buffer was added to dry DNA pellets. Pellets were resuspended for at least 1 min for each sample (to avoid bubbles). All components were kept on ice at all times. Water was included as a control sample. A fresh Eppendorf tube was used to make the Master Mix (MM) required for all of the samples. Initially, 70.4 ul of sterile distilled water was added to the Eppendorf tube. Then, 27.5 ul of 10X PCR buffer, 5.5 ul 10 mM dNTP, 16.5 ul 25 mM MgCl2, 15.4 ul Obe-F primer, 15.4 ul Obe-R primer, 13.2ul Kat-F primer, 13.2ul Kat-R primer, 11ul Thy-F primer, 11ul Thy-R primer, 16.5 ul Ala-F primer, 16.5 ul Ala-R primer, 13.2 ul Alba-F primer, 13.2 ul Alba-R primer, and 5.5 ul of Taq polymerase were added. The final volume in the Master Mix tube was 264 ul. This volume was good for 11 samples (2 extras) incase of spills or errors. Using a pipette, the MM was resuspended for1 min without making air bubbles to ensure everything was mixed. 24 ul of MM was pippetted into each of the PCR tubes. Each tube was made sure to have enough (spin down if not enough) before adding 1 ul of DNA or sterile distilled water to each tube. The DNA was resuspended, and 1 ul of it was added to each of the PCR tubes. For the control, 1 ul of water was added. We used a fresh tip each time. The PCR tubes were then placed on ice until it went into the PCR machine (X35). Once all the samples were ready, PCR tubes were placed in the PCR machine in the indicated grids. It was made sure that the machine was programmed with the required temperature to make many rounds of replication for the DNA with the primers. Refer to Table 1 for the required temperatures and times. The temperatures with the asterisk were repeated 40 times (40X) to amplify the target

DNA sequence. When the machine was done, tubes were frozen until they were used in the next step.

Table 1. Required temperatures and times for PCR cycle

Required temperatures	Required time
95°C	5 mins
*95°C	30 sec
*62°C	30 sec
*72°C	30 sec
72°C	5 mins
4°C	overnight
Freezer	Until the next visit

GEL ELECTROPHORESIS

Using a pipette, 5 ul of 6X loading dye was mixed with the PCR sample tube which contained 25 ul of sample in it (pipetting up and down). Initially, the DNA ladder was loaded into the first and seventh well. Then the full drop was loaded into the tip and 15 ul of sample was loaded into the gel. This was repeated for each sample using a fresh tip. It was made certain that the sample was going into the well and the pipette tip was not being jabbed into the agar. A fresh tip was used and mixed, and the subsequent sample was loaded until all samples were loaded. The gel ran at 120 V for 3 hours.

Results

Regarding DNA isolation, we observed small, white DNA pellets for each of our samples, which confirms that we were able to successfully derive our results from our DNA isolation (Figure 1). We were then able to proceed to the next step of PCR with our eight DNA pellets. During the PCR stage, we encountered an error with the MM containing tuna primers, so we had to make a new batch of MM and run the PCR machine a second time. We were successful on the second attempt with even volumes in the PCR tubes and were ready for the gel electrophoresis step.

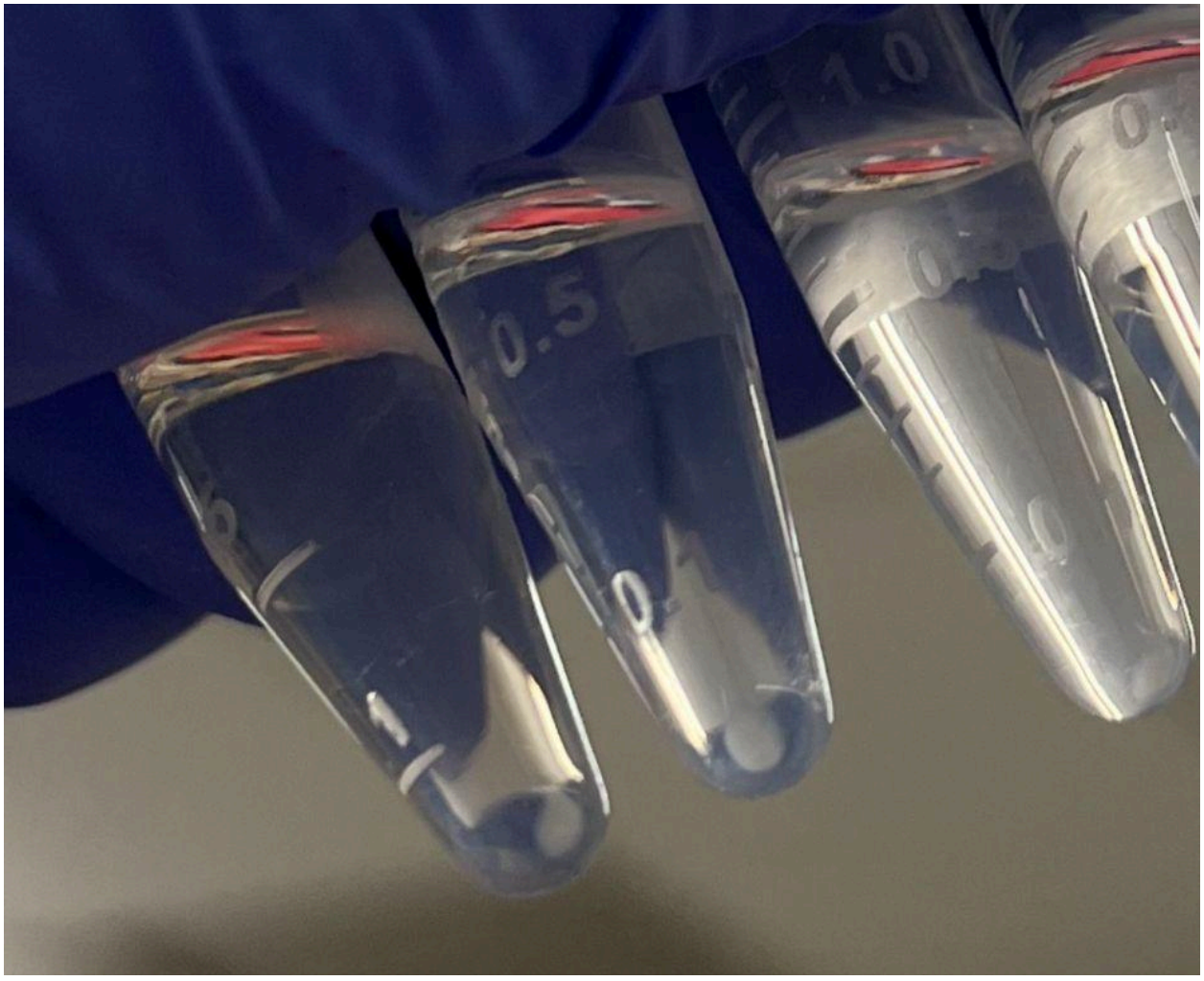

Figure 1. DNA pellets after DNA isolation. The pellets are the white spots at the bottom of the tubes with an Albacore and Yellowfin sample from left to right.

We observed banding patterns for four out of the eight samples during the gel electrophoresis. The gel ran for the full three to allow the solutions containing the loading dyes to seep through the small pores in the gel. Although, only half appeared. Three Yellowfin samples that gave results were compared with their expected banding patterns which matched up (Table 2). The one Albacore sample with results did not align with the expected banding pattern, hence was mislabelled and is a different type of tuna or fish species that we cannot confirm through this experiment.

			True species identification						
Product label	**Sample #**	**Source**	**Yellowfin (Ahi)** (127 bp)	**Skipjack** (238 bp)	**Albacore** (178bp)	**Bigeye** (270 bp)	**Bluefin** (200 bp)	**unidentified**	**No bands**
Raw Albacore	A1	Shizeny a (sushi)							****
Raw Yellowfin	Y1	Nesters Market	++++						
Raw Albacore	A2	Sushi Mugen							****
Raw Yellowfin	Y2	Save-On - Foods	++++						
Raw Albacore	A3	Bene sushi						- - - -	
Raw Yellowfin	Y3	Miyama e sushi							****

Raw Albacore	A4	Fujiya, Richmond							****
Raw Yellowfin	Y4	Superstore, Metrotown	++++						

Table 2. Results of gel electrophoresis for tuna samples. '++++' means a positive species identification. "- - - - " means an unidentified band. All samples were collected in Vancouver, BC except for A4. Blank cells means no DNA detected, and "**** " means no bands.

In Figure 2, three Yellowfin samples were observed to have base pairs (bp) around 127 while the Albacore sample also had a faint band around 127 bp, but there is not enough evidence to prove that it is another Yellowfin. The Albacore sample should originally have a band at 178 bp. No statistical analysis was done as this was an observational study.

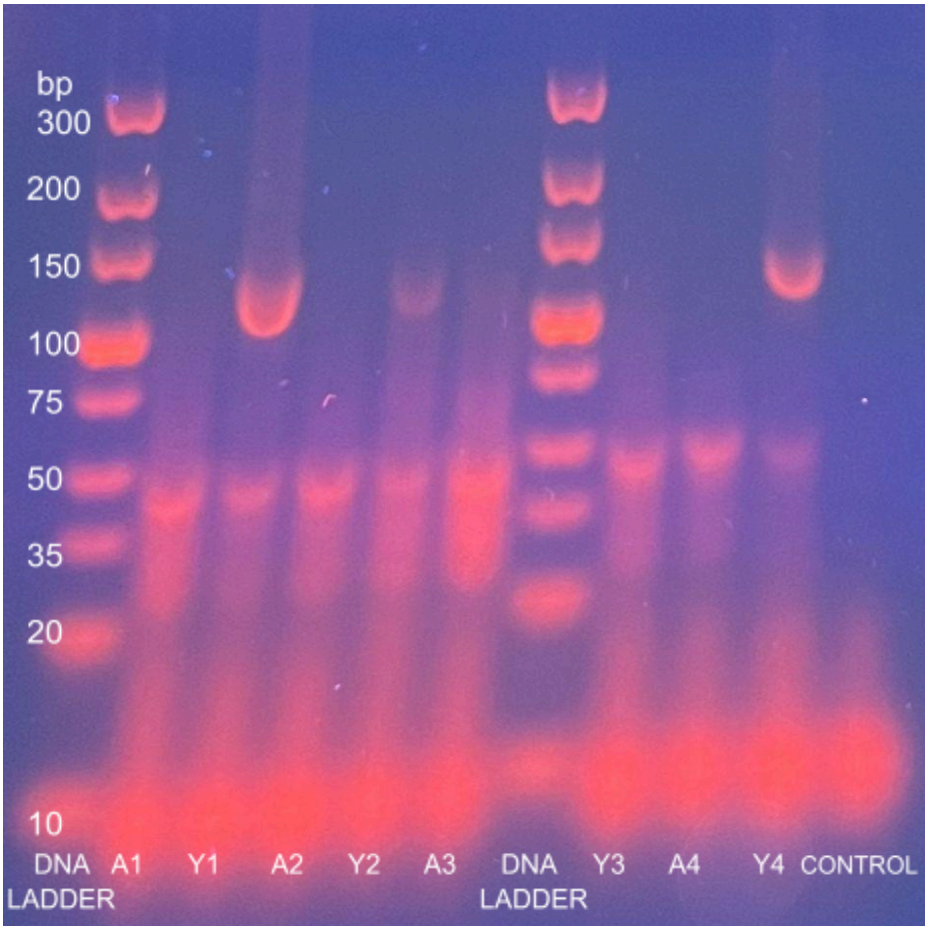

Figure 2. Results from gel electrophoresis, from left to right: DNA ladder, A1, Y1, A2, Y2, A3, DNA ladder, Y3, A4, Y4, control. Sample showing visible bands include Y1, Y2, A3 (very faint), Y4, all at 127 bp.

Discussion

Through this observational study, the results give insight into the transparency and accuracy of labelling in the raw tuna market. PCR was performed using 10 tuna-specific primers to help us quantify and analyze specific DNA sequences. Afterwards, gel electrophoresis was used to analyze the data obtained from the PCR. Based on the gel electrophoresis we were able to gather results from only 4 of our 8 samples. Our results showed the expected banding patterns for three of our four yellowfin samples which were Y1,Y2, and Y4, while Y3 did not show

visible banding results on the gel. Only one of our four albacore samples (A3) showed results while A1, A2 and A4 did not.

There could be many factors as to why half our results did not show in the gel electrophoresis stage. The reason could have started back at the PCR stage where the samples may have contained PCR inhibitors such as types of fat and protein that prevented the proper production of DNA copies (Thermo Fisher Scientific, n.d.). Another reason could be during the gel electrophoresis where the size of the DNA fragments may not have been sufficient for it to display on the gel, leading to no visible banding patterns (Lee et at., 2012). For the banding results that were visible, they revealed that all three yellowfin samples were labelled correctly and showed singular bands at 127 bp on the gel. However, the albacore sample did not show the expected banding pattern at 178 bp and instead seemed to show a very faint band also at 127 bp. This provides sufficient evidence to say that this albacore sample was mislabelled, and is not actually albacore. However it is not enough to say that it is actually yellowfin tuna. More investigation will be required to confirm. Based on the data from Oceana, we had originally thought that our tuna samples would be mislabelled as it was found that seafood samples in Halifax had a 38% mislabelling rate, with 71% of the substituted samples being cheaper species than what is labelled, such as yellowfin tuna being sold as bluefin tuna (Levin, 2018).

Yet, for the three yellowfin samples that showed results, they were all correctly labelled. As for the albacore, only one of our samples worked. It was mislabelled, but we can not draw any concrete conclusions as we would need to have at least one more sample to also be mislabelled to agree with a 43% mislabelling rate (Sotelo, 2018). The use of statistical analysis is disallowed due to the abundance of inconclusive data as well as it being an observational study.

Possible reasons as to why our gel didn't show results include errors during master mix making. The results shown on our gel were from a second attempt, after our first attempt where we failed to include all the primers. However we already added DNA to our PCR tubes with master mixes and proceeded with the rest of the PCR procedures. We then decided to perform PCR a second time, where the master mix was correctly made this time. We used the old DNA samples that were not thrown out. This may have contributed as our resuspension techniques may have resulted in uneven DNA distribution throughout the solution, and we ended up with less DNA in our master mixes for our second attempt. This could explain why our DNA ladders showed up fine, but some samples did not (Bennis, 2024).

The gel may also have not shown bands as it may not have been tuna at all. Escolar (Levin, 2018), one of the most commonly used fish types for substituting other fish, is illegal in many places over the world. This is because escolar is a fish that contains an oil made up of wax esters called gempylotoxin which is indigestible to humans, and can cause many health related reactions. These reactions may include diarrhea, nausea, vomiting, abdominal pain, and headaches (Canada, H., 2008). Escolar is used during mislabelling as it may be cheaper to obtain, so a higher profit can be made when sold as more expensive fish types (Canadian Grocer, 2021).

The number and variety of samples used in our study were limited. Here, we only tested samples that belonged to two different types of species (yellowfin and albacore) and from only 8 different locations overall. In the future, researchers may aim to examine several types of species as well as from several sources. Future research may try to assess the primers further than it was

assessed by the original author, Lee et al., (2022), to determine whether they would be eligible for a single PCR. This would offer better evidence of these primers reliability and sensitivity to concentrations of DNA.

Conclusion

The results indicate that tuna mislabelling is present, with three of the samples being correctly labelled according to species. This outcome supports our prediction that mislabelling exists within the raw tuna market and emphasizes the need for improved regulatory practices to ensure accurate labelling. By identifying species misrepresentation, our findings stress the importance of transparency and accuracy in tuna labelling for consumer trust and sustainability.

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Appendix

Table 1. Primers used in the multiplex PCR.

Target Species	Target Gene	Primer Name	Sequence (5′ → 3′)	Amplicon Size (bp)	Concentration (μM)	Accession No.	Reference
Thunnus obesus	ATP6	Obe-F	ACT TGC ATT CCC CCT ATG G	270	1.4	KY400011.1	This study
		Obe-R	GCT GTT AGG ATT GCC ACA G				
Katsuwonus pelamis	Cytb	Kat-F	GGT CCT AGC TCT TCT TGC A	238	1.2	NC_005316.1	This study
		Kat-R	TGC AAG TGG GAA GAA GAT G				
Thunnus thynnus	NADH5	Thy-F	AAC TCT TTA TCG GGT GGG AG	200	0.4	KF906720.1	This study
		Thy-R	[1] AGC GGT TAC GAA CAT TTG C**T**T C				
Thunnus alalunga	Cytb	Ala-F	GTT TCG TGA TCC TGC TAG TG	178	0.6	NC_005317.1	This study
		Ala-R	CCT CCT AGT TTG TTG GAA TAG AT				
Thunnus albacares	NADH4	Alba-F	CAT GAT TGC CCA CGG ACT TA	127	1.2	KM588080.1	This study
		Alba-R	TGT TGT TAT AAG GGG CAG C				

[1] The nucleotide sequence G was replaced with T (in bold).

Figure 3. Primers used for the PCR.

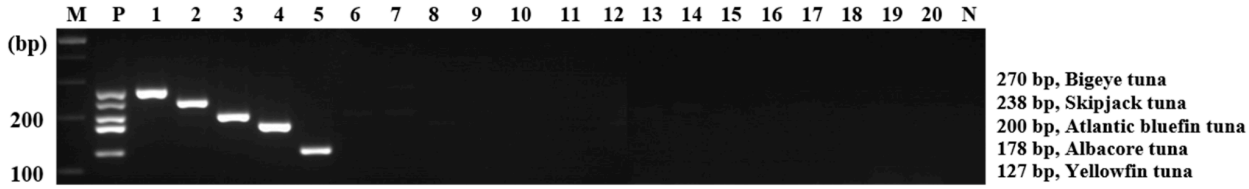

Figure 4. Specificity of Multiplex PCR. 1: Bigeye, 2: Skipjack, 3: Atlantic bluefin, 4: Albacore, 5: Yellowfin.