Analysis of Tuna Species for Identification of Proper Labeling Practices in Tuna-Containing Products

BIOL 342
Bhupesh Jassal, Daniel Lee, Anders Mirabee, Bill Yang

Abstract:

Numerous tuna-containing products were procured and tested via DNA isolation, polymerase chain reaction, and gel electrophoresis respectively. This was done in order to determine whether the labeled species of tuna is the species actually present in the product. Included among these samples were raw tuna, canned tuna, and tuna from a fast-food restaurant franchise for a total of five samples. These products were tested for DNA pertaining to the following species of tuna: Bluefin, Yellowfin, Albacore, Bigeye, and Skipjack. Our results yielded few conclusions as many of the products did not produce legible banding patterns on agarose gels after electrophoresis. This was probably due to metal contamination of the canned tuna samples. The fast-food tuna, which also returned inconclusive banding patterns, was in turn tested for DNA pertaining to livestock, including pig, cattle, goat, sheep, and chicken. All returned inconclusive results. However, the two raw tuna samples were identified as Yellowfin tuna and Ahi tuna (similar to Yellowfin and Bigeye tuna); both were labeled correctly.

Introduction:

Seafood is a staple of many diets across the globe. According to the Food and Agriculture Organization of the United Nations, approximately 17% of animal protein intake by humans is sourced by fish, and this amount is rising over time. However, while demand increases, supply remains constant in some cases and decreases in others due to overfishing (FAO). In response, many seafood vendors have taken to purposely mislabeled their products in order to account for the lack of supply for certain marine species.

This occurrence has been observed in multiple countries. For example, Bénard-Capelle et al. found that out of 390 samples of fish collected in France, 14 were mislabeled regardless of the reported species (mislabeling rate of 3.7%) (Bénard-Capelle et al., 2014). Christiansen et al. found that among 280 samples of seafood products procured in Brussels, Belgium, 87 were mislabeled by vendors (mislabeling rate of 31.1%), including 95% of all fish products said to contain Bluefin tuna, which is the most expensive species of tuna and was mostly substituted with other, cheaper species of tuna (Christiansen et al., 2018). Such circumstances necessitate rigorous testing of seafood products to ensure correct commercial labeling practices.

To this end, seeing as how mislabeling is a common occurrence with tuna products, we performed a Polymerase Chain Reaction (PCR) assay of five such products. Among these were canned Albacore tuna, canned Skipjack tuna, two samples of raw Yellowfin tuna (one of them labeled as Ahi tuna), and a sample of tuna from a fast food restaurant franchise. The purpose of this assay was to determine out of the above samples, which ones were labeled with the correct species of tuna, and which ones were mislabeled.

As most of our preliminary data pertained to samples procured outside of Canada (and particularly outside of British Columbia), we could not make an accurate prediction regarding the expected rate of mislabeling. Our small sample size (n = 5) compared to those of the preliminary data (n = 390 and n = 280, respectively) also contributed to our inability to make predictions. However, we did hypothesize that it is

quite unlikely that more than two of our samples would be mislabeled, as none of them were labeled as containing Bluefin tuna.

Methods

Part 1: DNA isolation

Place three replicates of 5 tuna product samples in Eppendorf tubes and mash them with toothpicks. Include water as a control sample. Add 300 uL of lysis buffer with proteinase K to each tube. Incubate tubes at 65ºC for 15 minutes and vortex them every 5 minutes. Place samples on ice for 5 minutes. Add 150 uL of protein precipitate reagent and vortex for 10 seconds. Centrifuge at max speed for 10 minutes, then transfer the sample liquids to new tubes without transferring the fat. Add 500 uL of ice-cold isopropanol to the new tubes and carefully invert them 40 times. Centrifuge at max speed for 10 minutes, then carefully pour off the isopropanol without disturbing the DNA pellets at the bottom of the tubes. Add about 500 uL of ethanol, and carefully pour it off without disturbing the DNA pellets. Add another 500 uL of ethanol and repeat pouring it off. Leave the caps open with the tubes on their sides at 25C overnight.

Part 2: Polymerase chain reaction

Add 30 uL of TE buffer to each of the dried DNA pellets. Add 172.5 uL of distilled water to fresh Eppendorf tubes. Then, add 75 uL of 50% glycerol, 15 uL of 10 uM forward primer, 15 uL of 10 uM reverse primer, 37.5 uL of PCR buffer, 7.5 uL of 10 uM dNTP, 30 uL of 25 mM Magnesium chloride, and 7.5 uL of Taq polymerase, in no particular order in order to create a master mix. Re-suspend the master mix, then add

24 uL of it to each PCR tube. Resuspend the DNA, then add 1 uL of it to each PCR tube. Add 1uL of water to an empty PCR tube to serve as a control.

Part 3: Gel electrophoresis

Add 1 uL of 6X loading dye to the PCR sample tube and mix with the pipette. Load the entire drop into the tip and load into the gel. Repeat for each sample with a fresh tip. Ensure the sample is going into the well. Use a fresh tip and mix and load the next sample until all samples are loaded. Run the gel at 120 V for 1 hour.

Part 4: Re-run of trials

The PCR and gel electrophoresis parts of the experiment were repeated for the Ahi tuna and the fast-food tuna. We produced a dH_2O control sample for both, and adjusted master mix amounts according to the number of trials being conducted (i.e., three for each sample).

Results

We derived visible DNA pellets for each replicate of our samples. This confirms that our first step of DNA isolation was performed successfully which we were able to use for our PCR and gel electrophoresis.

Due to errors associated with gel electrophoresis, we had to perform that particular step of the procedure twice; one with all of the five tuna species for which we tested (each with three replicates for a total of fifteen replicates) and one with only Ahi and fast-food tuna (each with three replicates for a total of six replicates). Furthermore,

we used meat primers for pig, chicken, goat, sheep, and cattle in order to check if any banding patterns would show since tuna primers did not return any conclusive results.

Though banding patterns did show for the fast-food tuna with meat primers, the bands were very densely packed and were below the 100 base pair line. Thus, since our expected banding patterns are all over 100 base pairs, we cannot confirm that it is in fact meat rather than contaminated tuna.

The PCR test was only able to identify DNA and show bands for six of our replicates successfully. These six replicates pertained to the Ahi and Yellowfin tuna samples. We compared the banding patterns produced by these replicates to the expected banding patterns (fig. 2) for their labeled species of tuna, then compiled the results into a table (Table 1).

			True species identification				
No	Product Type	Labeled species	Bigeye Tuna	Skipjack Tuna	Atlantic Tuna	Albacore Tuna	Yellowfin Tuna
1	Canned	Fast-food (Skipjack or Yellowfin)					
2	Canned	Fast-food (Skipjack or Yellowfin)					
3	Canned	Fast-food (Skipjack or Yellowfin)					
4	Raw	Ahi (Yellowfin /Bigeye)	+++++				+++++

5	Raw	Ahi (Yellowfin /Bigeye)	+++++				+++++
6	Raw	Ahi (Yellowfin /Bigeye)	+++++				+++++
7	Raw	Yellowfin					+++++
8	Raw	Yellowfin					+++++
9	Raw	Yellowfin					+++++
10	Canned	Skipjack					
11	Canned	Skipjack					
12	Canned	Skipjack					
13	Canned	Albacore					
14	Canned	Albacore					
15	Canned	Albacore					

Table 1. Results of PCR to commercial tuna products. '+++++' means a positive result.

Some sources report that Ahi tuna is simply the Hawaiian word used to refer to Yellowfin tuna, while others report that Ahi tuna is a unique species that shares banding pattern characteristics with both Yellowfin and Bigeye tuna. Per our gel electrophoresis results, we can see the Ahi tuna banding patterns do resemble both those of Yellowfin and Bigeye, at approximately 127 bp and 270 bp, respectively.

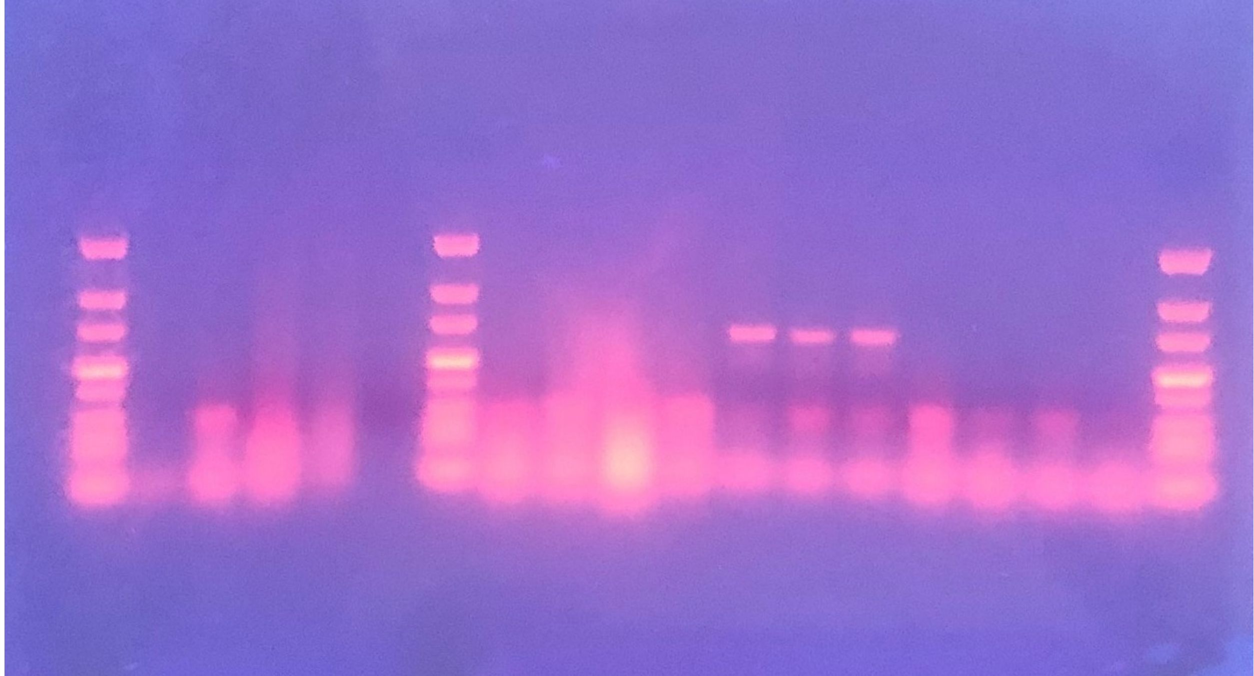

Figure 2. Bands result from gel electrophoresis attempt 1. From left to right: Ladder, Control, Albacore, Ahi, Ahi, Ahi, Ladder, Skipjack, Skipjack, Skipjack, Albacore, Yellowfin, Yellowfin, Yellowfin, Albacore, Fast-food, Fast-food, Fast-food, Ladder.

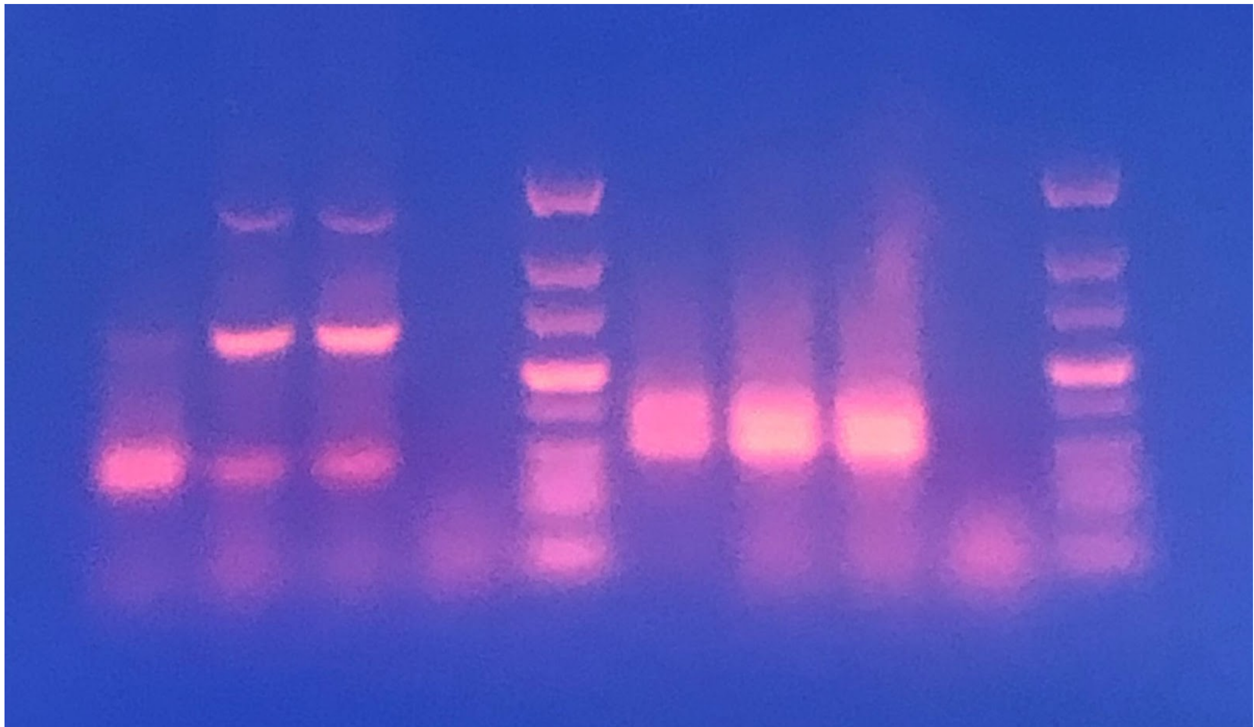

Figure 3. Bands result from gel electrophoresis attempt 2. From left to right: Control, Ahi, Ahi, Ahi, Ladder, Control, Fast-food, Fast-food, Fast-food, Ladder.

Discussion

In this study, we evaluate the labeling of tuna products with the ultimate goal of ensuring safety, quality, as well as transparency in the marketing of seafood products. Multiplex PCR was performed using 5 primers made to identify 5 common tuna species. Subsequent gel electrophoresis revealed that a large fraction of our sample trials failed to match the expected banding patterns for their respective tuna species. This may be due to many factors, including the novelty of the procedure used in this study. Ultimately, the presence of large amounts of inconclusive data disallowed the use of statistical analysis for mislabelling.

Our results showed the expected banding patterns for two of our samples, the Yellowfin and Ahi tuna samples, the latter of which is known to produce banding patterns reminiscent of both Yellowfin and Bigeye tuna. These two samples were also our two raw-form samples that had not undergone as much industrial processing as canned or fast-food tuna. The relative freshness of these samples may have contributed to our ability to derive legible banding patterns from them.

The samples for which bands were not produced consisted of two canned samples containing Albacore and Skipjack tuna, and one sample from the fast food restaurant franchise, which is reported by the company as either Skipjack or Yellowfin tuna.

It is possible that the results obtained from our canned samples are indeed due to the fraudulent mislabelling of these products. The multiplex PCR performed used primers specific to each of the five species of tuna, and would thus fail to amplify DNA

from an extraneous species (Lee et al., 2022). The absence of bands in these samples thus provides support to the possibility that the tuna belonged to a separate species, and mislabelling occurred. If not any of the tuna species, it is additionally possible that our samples belonged to other fish taxa altogether. A previous study by Oceana reported that tuna are highly mislabelled (59%) and that a large fraction of mislabelled tuna was correctly identified as an extraneous species (Warner, 2013).

However, since our inconclusive samples correlated perfectly to the use of highly processed canned tuna, one factor at play may be the inability of our novel procedure to account for industrial and commercial contaminants such as preservatives for canned tuna and condiments for fast-food tuna. With the use of canned tuna also arose the possibility that lipids, oils, or even metals from the container affected gel electrophoresis. Soaking samples overnight before the protocol has been known to remove lipids and oils, but this was not done in the present study.

Another possibility is that the primers used were over-specific in PCR, or to the DNA of the assigned species, and simply require further evaluation to be used reliably for Multiplex PCR. Other errors in our PCR, such as low Magnesium concentrations, or errors in our DNA samples, could have resulted in the increased specificity and our observed lack of results.

Regardless, here we elaborate on the use of tuna primers from the 2022 study conducted by Lee et al. while also working towards our main purpose of testing for mislabelling, a common occurrence in the seafood market.

Our study is limited in the number, and variety of samples used. Here, we tested samples labeled as belonging to two different types of products (processed and raw),

and four different species overall. Going forward, studies may aim to test multiple samples from each of the five species and any others for which reliable primers may be developed in the future. As for the primers used here, future studies beyond the originators, Lee et al. (2022), may aim to further evaluate these primers by testing for single PCR success after Multiplex PCR. This would provide better evidence for the reliability of these primers.

Conclusion

Overall, our experiment objective was achieved. Of our 21 tuna experimental trials, we were only able to identify that the Yellowfin tunas (including Ahi tuna) were properly labeled. We also identified three possible factors as to why three tuna samples (canned Albacore, canned Skipjack, and fast-food tuna) did not register banding patterns. The errors may be attributed to faulty primers, a new protocol that is unrefined, and/or metal contamination of the canned tuna species. For future directions, this experimental protocol may be optimized to reduce error and include more species samples and primers to broaden the scope. This may allow for statistical analyses to be conducted to identify possible patterns of mislabelling tuna species. If a certain company has multiple apparent mislabelling offenses, they can be approached directly for open conversation and collaboration towards the reasons for mislabelled tuna species.

Acknowledgments

We would like to acknowledge that we performed this assay on the traditional, ancestral, and unceded territory of the Musqueam First Nation. We would like to acknowledge Professor Celeste Leander, Tessa Blanchard, and the lab technicians for their support of this project.

Literature Cited

Bénard-Capelle, J., Guillonneau, V., Nouvian, C., Fournier, N., Le Loët, K., & Dettai, A. (2014). Fish mislabelling in France: Substitution rates and retail types. https://doi.org/10.7287/peerj.preprints.327v2

Christiansen, H., Fournier, N., Hellemans, B., & Volckaert, F. A. M. (2018). Seafood substitution and mislabeling in Brussels' restaurants and canteens. *Food Control*, 85, 66–75. https://doi.org/10.1016/j.foodcont.2017.09.005

Food and Agriculture Organization (FAO) of the United Nations, https://www.fao.org/state-of-fisheries-aquaculture/2020/en

Hawaii Seafood Organization. *Yellowfin Tuna (AHI).* *https://www.hawaii-seafood.org/wild-hawaii-fish/yellowfin-tuna/*

Lee, G. Y., et al. (2022). Multiplex PCR Assay for Simultaneous Identification of Five Types of Tuna (*Katsuwonus pelamis*, *Thunnus alalonga*, *T. albacares*, *T. obesus* and *T. thynnus*). *Foods*. *11*(3), 280.

Lee, Y. M., Lee, S., Hae-Yeong, K., (2021). A Multiplex PCR Assay Combined with Capillary Electrophoresis for the Simultaneous Identification of Atlantic Cod, Pacific Cod, Blue Whiting, Haddock, and Alaska Pollock, *Journal of Foods*. *10(11)*, 2631.

Warner, K., (2013). Oceana Study Reveals Seafood Fraud Nationwide. *Oceana*. 11.

Appendix

Target Species	Target Gene	Primer Name	Sequence (5′ → 3′)	Amplicon Size (bp)	Concentration (μM)	Accession No.
Thunnus obesus	ATP6	Obe-F	ACT TGC ATT CCC CCT ATG G	270	1.4	KY400011.1
		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
		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
		Thy-R	[1] AGC GGT TAC GAA CAT TTG CTT C			
Thunnus alalunga	Cytb	Ala-F	GTT TCG TGA TCC TGC TAG TG	178	0.6	NC 005317.1
		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
		Alba-R	TGT TGT TAT AAG GGG CAG C			

Figure 4. Primers used for the PCR

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