Something's Fishy?

DNA Barcode Identification of Fish Products From NYC Chinatown Markets

Dora Chen[1], Annie Chen[1], Milen Girvin[2] and Kathleen Nolan[3] (mentor)

[1]Eleanor Roosevelt H.S., [2]New Explorations into Science Technology and Math H.S., [3]St. Francis College

Funded by:

The Pinkerton Foundation

Abstract

As global demand for fish is at an all-time high, concerns about the integrity of seafood supply chains have emerged. In response, our project aims to investigate the prevalence of fish fraud, focusing on identifying patterns and factors contributing to mislabeling in the industry. In the process, we learned much about fish diversity, biology and fisheries. We purchased and sampled DNA from Pompano, Red Snapper, Belt Fish, Branzino, Porgy, Mud Carp, Tuna, Salmon, and Lemon Sole as well as various species of canned Tuna (Albacore, Yellowfin, and Skipjack) from Chinatown markets in Manhattan and Brooklyn to test the validity of their storefront representations. Through silica DNA isolation, amplification, and gel electrophoresis, we obtained PCR amplification to send out for sequencing. We obtained good DNA sequences from our Red Snapper and Pompano specimens. These sequences matched what we found in the NCBI known database through BLAST. From those results, we were able to compare and contrast DNA sequences and create phylogenetic trees through DNA Subway. Our fish are in the group Actinopterygii, the ray-finned fishes, so we instead portrayed a simpler phylogenetic tree that shows the relationships among the ray-finned fishes and other vertebrates.

Introduction

Undoubtedly, fish are in high demand in a world reliant on this nutritious sustenance (Tang et al. 2022). With this ever-rising pressure on global fish markets come strategies to preserve profitability in declining fish populations. Commercializing more valuable fish gives way for marketers to supply substitutions without the knowledge of the consumer. This leads to ambiguity and mistrust within patrons. Namely, in Guiyang, China, it was found that multiple fish products did not contain the respective species indicated on package labels (Tang et al. 2022). Similarly, in Mexico, and Australia, researchers discovered mislabeling and substitutes for wrongly marketed products (Munguia-Vega et al. 2022; Khalil et al. 2023). Heading southeast to the Coastal Amazon, researchers found additional fish species than marketed, even discovering a different threatened species (Rosas et al. 2018). In these case studies, the cytochrome c oxidase subunit I (CO1) gene (from the mitochondrial DNA genome) is used as a marker to refer to in taxonomically verified databases. These case studies highlight the economic favorability of false advertisements. However, this instills doubt in the consumers' perspective once discovered. For instance, in Alameda County, California, Subway gained a lawsuit in 2021 where Plaintiff Nilima Amin claimed that Subway violated their "100% tuna" policy, and other animal products were used (5, 6). These findings fuel skepticism and suspicion about what products truly circulate the market.

Additionally, we researched Ciguatera, a bacterium that causes poisoning and fish disease to spread through tropical marine fish carrying ciguatoxins that originate from dinoflagellates, small sea plants that grow around and on coral reefs. Ciguatoxins become more concentrated as the cycle of fish eating the ciguatoxins and then each other continues. Once infected with ciguatera, the toxins are impossible to get rid of, even through cooking. Ciguatera is often found in Barracuda, grouper, amberjack, red snapper, moray eel, hogfish, mackerel, surgeonfish, and parrotfish. People might avoid eating types of fish that they believe might contain ciguatera, which might skew the selection of fish to eat. Due to these health concerns, consumers acknowledge the dangers of purchasing these fish and, in turn, jeopardize the fish market. We find it important to raise awareness of the fish fraud that could occur to accommodate the possible profit loss and rightfully inform the public of the products they are receiving.

Thus, we decided to examine Chinatown markets, comparing the fish between a Manhattan location and a Brooklyn location. At the Brooklyn location, we observed three species of fish: Marbled Goby (*Oxyeleotris marmorata)*, Striped Bass (*Morone saxatilis)*, and Barramundi (*Lates calcarifer*). Upon further research, we learned that the Marble Goby is often found in Southeast Asia and lives in freshwater and brackish environments with little to no water movement. Interestingly, we also found that Marbled Goby is a delicacy in Asia, with many people believing it has healing properties after surgery and childbirth. Striped Bass are anadromous in America, meaning they live in the ocean but return to freshwater to spawn during the spring. Barramundi are found in the Indo-West Pacific, living in either freshwater or saltwater. All three fish are carnivorous. We examined different fish in the Chinatown Market: Red Snapper, Pompano, Red Snapper, Belt Fish, Branzino, and Porgy. Mud Carp, Tuna, Salmon, and Lemon Sole as well as various species of canned Tuna (Albacore, Yellowfin, and Skipjack) from additional stores in Brooklyn and Manhattan were studied. DNA was isolated from all samples, and the PCR procedure was used to amplify DNA that was visualized on a gel. Two positive PCR products were sent to GeneWiz for sequencing. After researching a scandal at the sandwich chain Subway, we also examined canned tuna. The famous fast-food chain Subway was lying about the tuna they served their customers. Upon investigation, the supposed "tuna" they were marketing was a mixture of different kinds of meat with little to no tuna at all.

Methods:

Fish were purchased in Chinatown, NYC, or I Brooklyn, NY (Table 1—provides additional information about the fish.)

DNA Isolation: To extract DNA from our samples for future analysis, we used the silica resin DNA isolation procedure. In a test tube, we added our specimen tissue samples with lysis solution. We will eventually separate the DNA from the protein and lipids. These steps involve lysis and centrifugation in which we will eventually get a DNA pellet. The silica resin helps in the separation process. The DNA will initially attach to the resin, and then we elute the DNA with a buffer. We eventually will obtain a DNA pellet. We will then wash and dry the DNA pellet. We will resuspend the pellet in buffer or sterile water.

DNA Amplification: Materials: DNA, Taq mix, Primer mix, Thermal Cycler. Next, we performed PCR (Polymerase Chain Reaction) with the collected DNA. First, we prepared a PCR tube with Taq mix and primer mix before adding a small amount of DNA to the PCR tube with a pipette. We then repeated this with all the DNA and placed the tubes in a thermal cycler. 94^{o}C for 2min, 94^{o}C for 15 sec., 54^{o}C for 15 sec., 72^{o}C for 50 sec., repeat from stop 2 34 times, cool down to 4^{o}C for infinity.

DNA electrophoresis: Agarose, gel box, gel tray, comb, rubber stoppers, 50X TAE buffer or 10X TBE buffer, power supply, (1%) loading dye

We made 2% agarose gel with a dye that stains the DNA. Heated the agarose in the microwave till it dissolved and no white flecks were seen. Let it cool and add it to a gel tray that has rubber stoppers at its ends, and a comb in position on the opposite end. Get rid of any bubbles with the tip of a pipette. After it has hardened (it will be transparent) we will pull out the combs. Two microliters of loading dye will be added to 8 ul of a DNA sample, the gel will be covered with a layer of buffer, and a current of 100 volts will be applied (first we will make sure that there is resistance in the form of amperes). The samples will separate and, hopefully, we will get a DNA product of the desired size. We set up a control with no DNA and ran it in the last lane.

DNA Sequencing:

There was DNA amplification so we sent our DNA products to GENEWIZ for DNA sequencing. The sequences were pasted into a blank box in the NCBI BLAST website, and the sequences were compared to known DNA sequences from fish species.

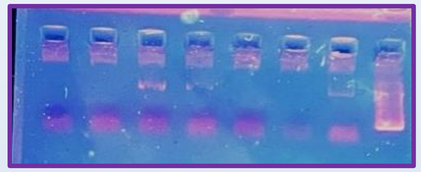


Figure 1. Gel depicting positive bands in 3, (4 replicate) and 7

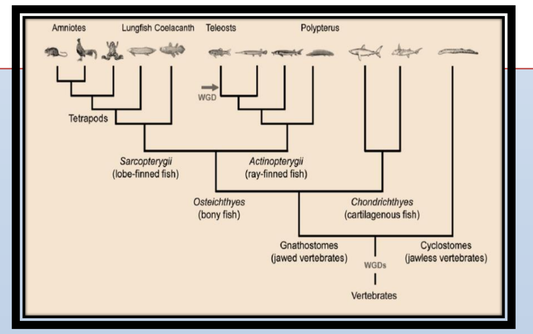
3F TRIMMED RED SNAPPER

CTAGTATTTGGTGCTGGGCCGGAATAGTAGGCACGGCCTAAGCCTGCTCATTCGAGCAGAGCTAAGCCAGCCAGGAGCTCTTCTTGGAGACGA
CCAGATTTATAATGTAATTGTTACAGCACACGCATTTGTAATAATTTTCTTTATAGTAATACCAATCATGATCGGAGGATTCGGGAACTGACTGA
TCCCATTAATAATTGGAGCCCCTGATATGGCATTCCCCCGAATAAATAACATGAGCTTTTGACTCCTCCCCCCGTCATTCCTGTTACTGCTCGCC
TCTTCTGGAGTAGAAGCCGGTGCTGGAACTGGGTGGACAGTCTACCCGCCCCTAGCAGGCAACCTAGCACACGCAGGAGCATCTGTAGACCTAAC
TATTTTCTCCCTACATCTGGCAGGTGTCTCCTCAATTCTAGGGGCCATTAATTTCATTACAACGATCATTAACATGAAACCCCCTGCCATCTCCC
AATATCAAACACCCCTATTCGTTTGAGCCGTCCTAATTACTGCTGTCCTACTCCTTCTCTCTCTGCCAGTTCTGGCGGCCGGAATTACAATACTT
CTCACGGACCGAAATCTAAACACAACCTTCTTTGACCCGGCAGGAGGAGGGGACCCCATCCTTTATCAACATCTGTTCTGATTCTTCGGNCACCC
TGAAGTGTCATAGC

7F TRIMMED POMPANO

TATCTAGTATTTGGTGCTTGAGCCGGATAGTAGGCAGCTTTAAGCCTGCTTATTCGAGCGGAGCTAAGTCAACCCGGCGCCCTCCTAGGGGAT
GACCAAATTTACAATGTAATCGTTACAGCCCACGCCTTCGTAATGATTTTCTTTATAGTAATGCCAATTATGATTGGAGGCTTTGGAAACTGACT
TATCCCACTAATGATCGGAGCCCCGGATATGGCATTCCCTCGAATAAATAACATGAGTTTTTGACTCCTACCCCCCTCTTTCCTTCTCCTTCTTG
CCTCCTCAGGGGTAGAAGCGGGGGCCGGAACAGGTTGAACAGTTTATCCCCCCTTAGCTGGTAATCTTGCCCATGCAGGAGCATCTGTTGATTTA
ACCATTTTCTCGCTTCATCTAGCTGGAATCTCATCAATTCTAGGAGCTATTAACTTCATCACAACAGTAATTAACATAAAACCTCATGCTGTATC
TATATATCAAATTCCACTATTTGTCTGAGCCGTTCTAATTACAGCCGTCCTTCTGCTCCTCTCACTTCCTGTTTTAGCCGCCGGCATTACAATAC
TTCTAACCGATCGAAACTTAAACACAGCCTTCTTCGACCCAGCTGGAGGAGGAGACCCCATCCTGTATCAACACCTCTTCTGANTCTTCGGNCAC
CCTGAAGTGTCATAGCNTGTTTCC

Figure 2. DNA sequences from two positive PCR products.

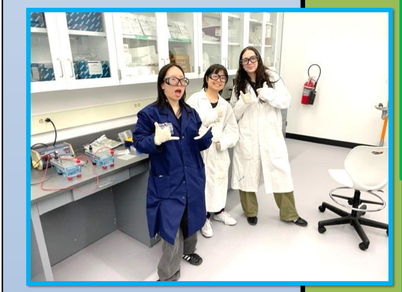


https://artpictures.club/autumn-2023.html

Figure 3. Phylogenetic tree of ray-finned fish (Internet)

Results:

We found two positives PCR products out of nine species analyzed (Figures 1 and 2). The two positives were from the red snapper and pompano samples from the Hung Kee Fish & Meat Market which means that they were correctly labeled. Both fish are in the group Actinopterigii, and we put them into a phylogenetic tree that is related to other fish species and additional vertebrates (Figure 3).



Picture	Common name	Species name	Range	Fishery/biology
	Belt fish	Trichiurus Lepturus	Belt fish are found in tropical and temperate regions in oceans throughout the world.	Belt fish is common in Asian markets.
	Red snapper	Lutjanus campechanus	Red snapper is found at 30 to 620 feet depths in the Gulf of Mexico, eastern coasts of North America, South America, and Central America.	Red Snapper is harvested off the Gulf of Mexico. It is considered one of the most delicious delicacies in the market. Its attractive coloring and appearance are what make it so popular.
	Pompano	Trachinotus	Pompano fish can be found in warmer waters ranging from Massachusetts to Brazil. They are most common in Florida's coasts and waters.	Pompano fishing is most popular in Florida. Pompano fishing is common all year round.
	Lemon sole	Microstomus kitt	Lemon sole is native to the shallow seas near Northern Europe.	The lemon sole does not taste like lemon and it is a flounder, not a sole.
	Porgy	Pagrus pagrus	Porgy is native to the western Atlantic, from Rhode Island to Bermuda and from the Gulf of Mexico to Brazil.	Porgy was so common on the East Coast that it was known as a trash fish.
	Mud carp	Cirrhinus molitorella	Mud carp is found in southern China and Vietnam.	Mud carp was a substitute for common carp after it was banned during the Tang Dynasty for having the same pronunciation as the emperor's family name.
	Flounder	Paralichthys dentatus	Flounder is found in the Gulf of Mexico and the Western Atlantic Ocean.	The recreational landings for flounder in 2022 was 8.6 million pounds.
	Skipjack tuna	Katsuwonus pelamis	Skipjack tuna are found in tropical, subtropical, and warm temperate waters of all oceans. In the western Atlantic, they are mostly found from Massachusetts to Brazil, including the Gulf of Mexico and the Caribbean.	Skipjack is the most common of the main commercial tunas, and its population is considered sustainable against its current consumption.
	Albacore tuna	Thunnus alalunga	This is a cosmopolitan fish, occurring in tropical waters of all oceans. At least two stocks (northern and southern) exist in both the Atlantic and the Pacific oceans, with little or no interchange across the warm equatorial waters.	Albacore tuna is one of the most sought-after fish around the world, both commercially and recreationally, and is classified as a Highly Migratory Species.

Table 1. Fish that we analyzed.

Discussion

We found two positives out of nine species. We found out that they were correctly labeled (red snapper and pompano). We are not sure why we did not get additional positive results—perhaps freezing or other handling of the DNA. We did not get positive results from the canned fish, possibly because the DNA was degraded through cooking. The other fresh fish most likely yielded null results due to the fish being frozen for too long, or, perhaps the fish underwent a frozen-thaw-frozen cycle, in which the DNA could have become degraded.

Had we continued this project for longer, we would have sampled more fish from other regions of the world and focused more on fresh fish than frozen and cooked. Detecting and looking for fish fraud is important because it can have significant impacts on the economy, human health, and society. Additional projects might include seeing if we can detect Ciguatera in fish and examining the local diversity of fish obtained from the Hudson and East Rivers through seining.

References:

Khalil, A. M., Gainsford, A., & van Herwerden, L. 2023. DNA barcoding of fresh seafood in Australian markets reveals misleading labeling and sale of endangered species. Journal of Fish Biology. 102(3):727–733.

Munguia-Vega A, Terrazas-Tapia R, Dominguez-Contreras JF, Reyna-Fabian M, Zapata-Morales P. 2022. DNA barcoding reveals global and local influences on patterns of mislabeling and substitution in the trade of fish in Mexico. PLoS ONE.17(4):1-22.

Rosas U, Menendez F, Cornejo R, Canales R, Velez-Zuazo X. 2018. Fish DNA barcoding around large marine infrastructure for improved biodiversity assessment and monitoring. Mitochondrial DNA Part A. 29(8):1174-1179

Tang Q, Lui Q, Duan Q, Deng L, Zhang R. 2022. DNA Barcode Identification of Fish Products from Guiyang Markets in Southwestern People's Republic of China. Journal of Food Protection 85(4):583-590.

Acknowledgements

We would like to acknowledge Allison Mayle and all the other people at the CSH DNLC who help us with our project, the Pinkerton Foundation, and St. Francis College