Authors: Nicholas Eng and Brian Shi Mentor: Dr. Vanaja Zacharopoulos Trinity School New York

Abstract

L.campechanus (northern red snapper), is found in the Atlantic Ocean and parts of the Gulf of Mexico. Red snapper has been identified as the most mislabeled fish in the United States with 87% of the samples being mislabeled and, as a result, is subjected to variable pricing. In order to verify the authenticity of the red snapper sold in New York City, we purchased fish labeled as red snapper from six fish markets across three different boroughs: Manhattan, Brooklyn, and Queens. We hypothesized that there would be mislabeling of red snapper sold in stores throughout New York City. We tested the samples using DNA barcoding to determine the species specificity. We found that four of the fish samples analyzed were various species of snapper such as Lutjanus purpureus, Lutjanus peru, and Lutjanus synagris. However, two of the fish samples were not. One of the two was Sebastes sp., the common rockfish, and the other was Lates niloticus, the nile perch. None of the samples were Lutjanus campechanus, which is known as northern red snapper. This confirmed our hypothesis that the mislabeling of fish especially red snapper is widespread across different boroughs in New York City. The substitutes are often cheaper in both quality and pricing and are usually of the lesser environmentally sustainable species. This also raises the question of whether the difference among these various species of snappers justifies the wide range of prices they are being sold at.

Introduction

Fish fraud has been a well-documented problem throughout the United States. Attorney General Barbara D. Underwood recently released a report: Fishy business: seafood fraud and mislabelling in New York State supermarkets which reported that more than 25% of seafood purchases were mislabelled. While the mislabelling was in every seafood category, it was a particularly significant problem with red snapper, lemon sole, and grouper. Furthermore, although mislabelling occurred throughout New York State it was especially prevalent in New York City where the mislabelling rate was found to be an astounding 42.65%. For those who incorporate seafood into their daily meals, fish plays an

important role in a person's diet and health. Many species of fish are rich in vitamins, proteins, and healthy fats. There are many species of fish consumed in New York City with different characteristics and flavors and availability, leading to a range of different prices.

Red snapper has a very distinct appearance, with a pinkish-red body and a triangular snout. Yet, when red snapper is portioned into fillets valued at over ten dollars per pound, its appearance resembles tilapia or sea bream flesh, both sold for an average of two dollars per pound. Since the 1840s, the northern red snapper has been harvested from around the Gulf of Mexico. In the early development of these fisheries, the harvest of the red snapper was limited because it was severely overfished. Due to the restricted number of red snapper that can be fished, the price of this type of fish is quite high ranging from the species and size. As a consequence, some fish markets, groceries, and restaurants have resorted to replacing red snapper with other, less expensive, varieties of fish that appear similar to red snapper. Once whole fish are portioned into fillets, they are less recognizable to the consumer, making it easier to substitute a cheaper fish.

Our team was interested in determining if the varied prices of fish sold as red snapper corresponded to their genetic authenticity. Using DNA Barcoding, we have genetically identified fish labeled as red snapper from multiple stores in New York City and compared the results to DNA sequences of genuine northern red snapper. We suspected that not all of our samples were red snappers due to the wide range of pricing from a mere two dollars to high of seventeen dollars.

Fig 5 Differences in morphology between the snapper species analysed.



Mislabelled Red Snapper in NYC

Materials & Methods

We selected whole fish that were sold as snapper or red snapper. We purchased our samples from small neighborhood grocery stores and portioned into small chunks and stored the tissue in a -20°C freezer until DNA extraction. All samples were processed for DNA barcoding following the protocols provided by the Urban Barcode Project. We used the Wizard Genomic DNA Purification Kit from Promega for DNA extractions. Extracted DNA was quantified using Nanodrop 1 spectrophotometer. Sufficient DNA was obtained from each sample. The DNA was amplified using fish CO1 gene primers. An approximately 655bp segment was amplified and subsequently subjected to 2% agarose gel electrophoresis. If DNA bands were observed in the relevant size location the samples were sent out for sequencing to Genewiz. Sequencing results were analyzed using DNASubway (<u>https://dnasubway.cyverse.org/</u>) for bioinformatic evidence.

Sample ID	BLAST Results	Bit Score	e	Mismatches	% Corresponding
KJH-001 (TS1)	L. purpureus	1180	0	0	85.42
KJH-002 (TS2)	L. purpureus	1180	0	0	90.57
KJH-005 (TS3)	L. synagris	1182	0	0	93.74
KJH-003 (TS5)	L. peru	1182	0	0	99.17
KJH-007 (TS4)	Sebastes sp.	1159	0	4	95.64
KJH-008 (TS6)	Lates niloticus	1177	0	0	92.11

- + ATCG		
Sequence Conservatior	1	
Sequence Variation		
Consensus		
1. KJH_002_1 - F	Ì	
2. KJH_001_1-F		
3. KJH-005		
4. KJH-003		
5. KJH_001_2		
6. KJH_002_2g 3		

Fig 1 Summary Data Table



Fig 2 Gel electrophoresis of PCR CO1 amplified segment of fish DNA









L. purpureus (\$16.99/lb)

L. purpureus (\$9.99/lb)

L.peru (\$7.99/lb)

Sebastes sp. (\$5.99/lb)

Results

None of our samples were positive for Lutjanus campechanus, the name for the northern red snapper. However, three of the six species detected were species of the Lutjanus genus as determined by amplification of the CO1 (cytochrome oxidase 1) mitochondrial gene (Fig2). Interestingly, from our small sample selection, we were able to find three different species of Lutjanus; L. purpureus (southern red snapper), L. peru (Pacific red snapper) and L. synagris(lane snapper or bream) (Fig 3 and Fig 4). Morphological observations of the whole fish showed significant differences in size, color, markings, and price per pound (Fig 5). We were especially surprised when we discovered two of our samples to be Sebastes sp and Lates niloticus. While the flesh of the Sebastes sp and Lates niloticus resembled that of the red snapper, the results did not match.









L. synagris (\$1.99/lb)

Lates niloticus (\$13.99/lb)

According to NOAA Fisheries, red snapper is defined as L. campechanus with common names like northern red snapper or genuine red snapper. The data obtained from our study surprised us, as we did not anticipate the presence of such a large variety of snapper species sold in local grocery stores and fish markets. Our hypothesis that genuine red snapper fraud or mislabelling is still rampant was proved correct. None of the samples analyzed were northern red snapper (L. campechanus), not even the most expensive sample obtained. Given the widespread publicity that fish fraud receives, it is somewhat surprising that the broad labeling of this species is still prevalent. Although we only analyzed limited samples of red snapper, the findings from our study are consistent with the recent New York State Attorney General's report on fish mislabeling. If anything, our study suggests that the problem is even more prevalent than detailed in the report. There are three major problems with fish mislabelling: 1. Substituting red snapper with a cheaper fish cheats consumers who believe they are paying for the more expensive red snapper. 2. Cheaper fish varieties may be of less nutritional or even dangerous. For example, lane snapper has a higher mercury level than red snapper. 3. The fish vendors may also be unaware of the variation in fish species sold to them as genuine red snapper. While we purchased whole fish that resembled and was sold as red

snapper, we noticed the pricing of the different species varied greatly, from \$2 per pound to \$17 per pound. The range of pricing may be the result of species differences; however, the morphological differences in color and size were also quite varied. We still found it to be incredulous that we were unable to find a single sample of the species we were looking for, Lutjanus campechanus. An interesting finding was that our one fillet sample was found to be L. niloticus (Nile perch), a completely different genus. In addition we also obtained a sample of fish that turned out to be Sebastes species (rockfish species).

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CSH Cold Spring Harbor Laboratory DNA LEARNING CENTER

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Fig 5 Phylogenetic tree showing sequence relationships between samples

Discussion

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