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Prescreening veterinary drug residues, heavy metal concentration, and genetic authentication in retail catfish fillets in the Northeast United States

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ABSTRACT

Veterinary drug residues, heavy metals, and species authentication were performed on the demand of the regulatory agency in retail catfish fillets. A total of 362 domestic and imported catfish samples collected from various retail markets in the Northeast region of the United States were tested for the presence of chloramphenicol (CAP), malachite green/gentian violet (MG/GV), As, Cd, Pb, and Hg according to the USDA - FSIS protocols. Species identification and mislabeling were also evaluated by DNA barcoding. Results showed 94.2% of samples barcoded successfully and an overall of 11.1% mislabeling was detected. The highest mislabeling rates were estimated for *Pangasius bocourti* (100%) and *Pangasianodon hypophthalmus* (16.7%) by species and for Cambodia (45.5%) and Vietnam (19.3%) by country of origin. Eleven samples (0.8%) tested presumptive positive for CAP while MG/GV was detected in 80 samples (22.1%). A total of 49 catfish samples (13.5%) were found to have detectable levels of heavy metals. Cd was found in 44 samples (12.2%) while 4 samples had Pb (1.1%) and only one sample had a detectable level of Hg. The concentration of Pb ranged from 35 μg kg^{-1} to 281 μg kg^{-1} and varied from 10.09 μg kg^{-1} to 395 μg kg^{-1} for Cd. Our results provide basic information about the mislabeling rate and the quality of retail catfish fillets in terms of veterinary drug residues and heavy metal concentration in the Northeastern United States which suggest the necessity of a monitoring program for seafood authenticity and quality.

1. Introduction

Catfish farming is the dominant domestic aquaculture in the United States (Rabbani et al., 2011). It has contributed to 47% of aquaculture production (NOAA, 2011) and represented 69% of food sale in 2005 (USDA-NASS, 2006). Due to its relished taste, the market demand for catfish products is increasing consistently in the US, as the total sale of catfish for human consumption increased ~11% from ~US$340.6 million in 2012 to ~USA$379.2 million in 2019 (USDA-NASS, 2013, 2020). The market does not confine to domestic production anymore and seafood industries have responded to the growing demand by importing products mainly from Vietnam, China, and Thailand (Lee & Kennedy, 2010; NMFS, 2011; USDA-NASS, 2011). This brings a variety of new challenges into the context such as quality control, food safety, mislabeling, human rights, etc. (Borit & Olsen, 2012; He, 2018; Lee & Kennedy, 2010). To regulate the market, the US national authorities need to implement new legislation (e.g.: COOL, COGP) aimed towards protecting public health, and supporting the business to thrive (Federal Register, 2003; Harvey & Blayney, 2002; Leal et al., 2015; Muhammad et al., 2010). Creating baseline data of catfish quality and authenticity then seems necessary to guarantee the aforementioned goals to ensure seafood safety (Tacon et al., 2010; Trienekens & Zuurbier, 2008).

Many studies have confirmed mislabeling in the US catfish market (Consumer Reports, 2011; FDA, 2012; Wang & Hsieh, 2016). *Pangasius* sp., for example, is usually substituted for more expensive fish species and is one of the most mislabeled fish in the market, 18 species have been enumerated so far (Warner et al., 2016). DNA barcoding has been found to be an effective technique for solving controversies related to species identification, food safety, and food fraud (Filonzi et al., 2010; Hubert et al., 2008; Jacquet & Pauly, 2008; Nicole et al., 2012; Steinke et al., 2009; Teletchea, 2009; Wong & Hanner, 2008). The method involves the use of mitochondrial cytochrome *c* oxidase subunit I (COI) as a complementary tool in species identification (Hebert et al., 2003; Nneji et al., 2020). According to Christiansen et al. (2018), DNA

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barcoding successfully detected an overall 31.1% mislabeling in Brussels' seafood restaurant including cod (13.1%), sole (11.1%), bluefin tuna (95%), and hake (71.43%). Also, DNA barcoding distinguished mislabeled catfish in 12.5% of catfish dishes in California (Bosko et al., 2018), 27% of restaurant dishes in the Southeastern US (Wang & Hsieh, 2016), and 14.3% of restaurant samples in the Northeastern US (Consumer Reports, 2011). However, limited information is available on mislabeling catfish fillet collections from retail US markets (Bosko et al., 2018).

Food safety, meaning being confirmed to not have contaminants such as pathogens, heavy metals, and drug residuals beyond the permissible levels (Cole et al., 2009; Sapkota et al., 2008), becomes one of the serious customer concerns (Claret et al., 2014; Ghisi & de Oliveira, 2016; Kole et al., 2009). In recent years, there has been numerous evidence showing that catfish from overseas failed to comply with rules on veterinary drug residues (Barboza, 2007). Asian seafood products have been reported to violate the veterinary drug residues standards frequently, especially domestic products from Vietnam (Love et al., 2011, pp. 7232–7240). For example, *Pangasius* catfish farmed in Vietnam and Malaysia have been detected as presumptively positive for antibiotics and malachite green and leucomalachite green residues, respectively (Kwan et al., 2018; Rico et al., 2013). Uchida et al. (2016) also detected 8 antibiotic residues in farmed fish and shrimps in Vietnam including quinolones (ciprofloxacin, enrofloxacin, norfloxacin, ofloxacin, and oxolinic acid), sulfonamids (sulfamethazine, sulfamethoxazole), and trimethoprim. Similarly, the occurrence of 20 veterinary drug residues were detected in domestic fisheries products farmed in South Korea in 22.7% of samples, mostly fluoroquinolones, enrofloxacin, tetracyclines, and oxytetacyclin (Kang et al., 2018). Despite the negative effects heavy metals have on public health, plenty of recent researches alarmed heavy metal contaminations in domestic and imported farmed catfish (Ferrantelli et al., 2012; Molognoni et al., 2016; Ozbay et al., 2013; Reham, 2012; Santerre et al., 2001a).

Given that the quality of aquaculture production can be assured by controlling safety, authentication, freshness, and traceability (Freitasa et al., 2019), seafood products need to be monitored continuously. The present study was funded by the United States Department of Agriculture-Food Safety Inspection Services (FSIS) OCIP to create baseline data of catfish quality in the Northeastern United States. Therefore, this study was carried out to first: identify fish species using DNA barcoding of the retail catfish fillets, second: verify mislabeling based on the concordance with labels of the fillets, and third: assess the quality of fillets concerning the presence of veterinary drugs and concentrations of heavy metals. Since there is little data available on the quality and authenticity of catfish market in the Northeastern United States, our results will represent a detailed report to help the local managers evaluate current inspection policy and monitoring program.

2. Materials and methods

2.1. Sample collection

The retail farmed catfish samples were collected from different regions in the Northeast United States including; Pennsylvania, New York, Delaware, Maryland, and Virginia over a period of 11 months from November 2010 to September 2011. The samples were purchased from 15 markets, an average of 3–4 markets per state, including grocery stores and retail fish markets which were randomly selected and shopped biweekly from the fish frozen section of the store or from the seafood counter on Sundays. Catfish, swai, basa, and tra were the species sought as samples, knowing that they are the most common names labeled for the catfish fillet in the US market (Watts & Hymel, 2018). A sample consisted of five individually fish fillets. All the catfish fillets purchased from grocery stores were subject to the law of Country of Origin Labeling for Fish and Shellfish (COOL, 2009). For each sample, country of origin was assessed by examining the packaging labels as well as all relevant signage (e.g., placards, tags, signs, etc.) at the point of sale (Bosko et al., 2018). If the information was unavailable, then the shopper asked the employee behind the counter as to the country of origin for the catfish sample. The shopper was also trained to examine the surface of the retail case upon which the fish was placed and to observe if the employee wore gloves to pick up the fish from the case. Also, the shopper was instructed to tell the employee to pull fish in the case from the top, middle, and bottom of the pile of fish in order to obtain a random selection. All details about each sample were recorded, for example, the labeled name and preservation status (fresh, thawed, and frozen). The samples then were transferred to the Catfish Inspection Laboratory in Delaware State University using a cooler contains ice packs with a thermometer (4 °C). Only one shopper involved in sample collection from the stores.

A total number of 362 frozen/fresh catfish samples were then transferred to the Catfish Research Laboratory at Delaware State University (DSU) for chemical testing of veterinary drug residues and heavy metal contaminants. Replicates of the fillet samples were also shipped to the Aquatic Genetics and Genomics Laboratory at Auburn University (AU) and stored at −20 °C for further genetic authentication experiments. Regarding the samples' labels, they originated from the US (n = 199), Vietnam (n = 76), China (n = 23), Thailand (n = 10), and Cambodia (n = 10). However, 44 samples were not assigned to a specific country (Not Known).

2.2. Sample preparation for chemical analysis

Each retail catfish sample included five individual fillets that were finely blended using a generic blender (KitchenAid, Benton Harbor, MI). The subsamples were prepared according to Ozbay et al. (2013), to avoid contamination with external agents. Subsequently, samples were kept at −20 °C until they were tested.

2.3. DNA barcoding

2.3.1. Tissue lysis and DNA extraction

Total genomic DNA of semi-thawed fish muscle (50–100 mg) was extracted and purified using the DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA) following the manufacture protocol with the following modification: 600 μl of Qiagen Cell Lysis Solution and 5 μl of Proteinase K (at 20 mg/ml) was added to each sample and incubated at 55 °C for 14–16 h. The lysate was mixed with 600 μl of Qiagen Protein Precipitation Solution and centrifuged at 16,000 g for 3 min. Then, 600 μl of 100% isopropanol and 600 μl of 70% ethanol were used to wash the DNA pellet during consecutive centrifugation steps at 16,000 g for 2 min. Finally, the DNA pellet was diluted with 20–100 μl of TE buffer and quantified in an Ultrospec (model) spectrophotometer (Biochrom, Cambridge, United Kingdom) by evaluating the ratio A260nm/A280nm (Handy et al., 2011). Agarose gel 1% with ethidium bromide was also used for electrophoretic analysis.

2.3.2. Polymerase chain reaction (PCR) and cleanup PCR

Pairs of primers were used to amplify a region of the cytochrome *c* oxidase subunit I (COI) of DNA extracted from samples which are summarized in Table 1. The reaction was carried out in 10 μl volumes containing 3.7 μl of ultrapure DNase/RNase-free distilled water, 1 μl of 10x DNA polymerase buffer, 0.4 μl of 50 mM MgCl2, 0.8 μl of 10 mM dNTP mix, 1 μl of each 10 μM forward and 1 μl 10 μM reverse primer cocktail (Table 1), 0.1 μl of Invitrogen Platinum Taq DNA Polymerase 5U/μl, and 2 μl of DNA template. PCR thermo-cycling started with 2 min at 94 °C, continued with 40 cycles of denaturation at 94 °C for 30s, annealing at 52 °C for 40s, and extension at 72 °C for 1 min. The program ended with 10 min at 72 °C and hold at 4 °C (Wong & Hanner, 2008).

To purify the amplification product, a PCR cleanup treatment was performed in a final volume of 12 μl mixture consisting of 5.3 μl nanopure water, 0.7 μl Exonuclease I buffer 10x, 0.5 μl Exonuclease I 20U/μl

Table 1
Primer pairs used for amplification and sequencing of the COI region. M13 primers and M13 tails are denoted in gray.

Name	Primer 5'-3' sequence	Cocktail name
C_FishF1t1; VF2_t1	5'TGTAAAACGACGGCCAGTCAACCAACCACAAAGACATTGGCAC3'	Combined to cocktail F1+1 (ratio 1:1)
C_FishF1t1; FishF2_t1	5'TGTAAAACGACGGCCAGTCGACTAATCATAAAGATATCGGCAC3'	
C_FishR1t1; FishR2_t1	5'CAGGAAACAGCTATGACACTTCAGGGTGACCGAAGAATCAGAA3'	Combined to cocktail R1+1 (ratio 1:1)
C_FishF1t1; FR1d_t1	5'CAGGAAACAGCTATGACACCTCAGGGTGTCCGAARAAYCARAA3'	
M13F	5'TGTAAAACGACGGCCAGT3'	M13F or M13R
M13R	5'CAGGAAACAGCTATGAC3'	

(New England Biolabs, Hitchin, United Kingdom), 0.5 μl rAPid Alkaline Phosphatase 1U/μl (Roche, Penzberg, Germany), and 5 μl of the PCR product. The mixture was incubated at 37 °C for 30 min and denatured at 80 °C for 20 min in a PCR thermal cycler PTC 200 (BioRad, Hercules, CA). Final PCR amplification products (a 652 bp region of the COI gene) were checked on a 1–2% agarose gel, visualized with ethidium bromide staining on a BioRad Molecular Imager® GelDoc™ XR system.

2.3.3. Cycle sequencing reaction and cleanup sequencing

Bidirectional sequencing PCR was completed via ABI BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Waltham, MA) according to the instruction provided by the manufacturer. To remove the unincorporated dye terminator prior to electrophoresis, the ethanol/EDTA precipitation method was applied to the sequencing PCR product. After purification, the product was loaded into the ABI 3130xl Genetic Analyzer for capillary electrophoresis using the ABI POP7 polymer and a 50 cm capillary array. All sequences then were compared with available sequences in Barcode of Life Data System (BOLD) databases using Sequencing Analysis 5.2 (Gene Codes Corporation, Ann Arbor, MI) as well as cross-checked with submitted samples in NCBI's GenBank and internal known controls. Mislabeling was detected when the species name that appeared on the label of retail catfish samples did not match with the genetic confirmation output (Christiansen et al., 2018).

2.4. Drug residue and heavy metal contaminants

2.4.1. Chloramphenicol (CAP)

Detecting the presence of CAP in homogenized catfish samples was accomplished with the help of TRANSIA Plate Chloramphenicol Test Kit (BioControl, Bellevue, WA) in a competitive ELISA based in-vitro experiment as described in USDA-FSIS CLG-CAM1.01 protocol (USDA-FSIS, 2010d). For analysis, 3 ± 0.1 g of homogenized catfish samples was weighed. The samples were analyzed for CAP in duplicates and the minimum concentration of CAP that can be measured in the ELISA technique was 0.25 μg kg^{-1}.

2.4.2. Malachite green/Leuco malachite green (MG/GV)

The 2 ± 0.1 g of homogenized catfish samples underwent an ELISA analysis in which the presence of malachite green (MG)/leuco-malachite green (LMG) and crystal violet (CV)/leuco-crystal violet (LCG) was screened via Malachite Green/LMG ELISA Test Kit (BioScientific, Austin, TX) according to USDA-FFSIS CLG-MG/CV3.00 protocol (USDA-FSIS, 2010e). This method is not efficient to measure the concentration of the four compounds separately but can detect a total commutative concentration of MG, LMG, CV, and GV as low as 1 μg kg^{-1}. Similar to CAP screening, the experiment was conducted in duplicates.

2.4.3. Heavy metal analysis

Digestion procedure and preparation of blank and standard solutions were followed as described in USDA protocols for Arsenic (As, USDA-FFSIS CLG-ARS1.04), Cadmium (Cd, USDA- FSIS CLG-TM1.01), and Lead (Pb, USDA- FSIS CLG-TM1.01) (USDA-FSIS, 2010c; 2010a). The absorbance of each metal was recorded by AAnalyst 600 Atomic Absorption Spectrometer (PerkinElmer, Waltham, MA) at the wavelength of 193.7, 228.8, and 217.0 nm respectively. The relative concentration of the elements was calculated using the calibration curves for three replications (Babu & Ozbay, 2013).

Mercury (Hg) analysis was done according to USDA- FSIS CLG-MERCI1.00 protocol (USDA-FSIS, 2010b). The absorbance was read on the digital screen of the FIMS 100 Flow Injection Mercury System (PerkinElmer, Waltham, MA) at the wavelength of 253.7 nm. The concentration of Hg was later estimated in triplicates relative to the calibration curves (Babu & Ozbay, 2013).

2.5. Statistical analysis

The efficiency of DNA barcoding in species identification of the retail catfish fillets in the present study was checked by estimating the barcoding success rate and percentage of BOLD matching. Also, comparing the commercial label and genetically identified catfish species accounted for mislabeling occurrence.

The data on heavy metal contamination were evaluated for normality and homoscedasticity before statistical analysis using normality (Shapiro–Wilk) and equal variance test (Levene). Since our data violated the normality assumption, a *t*-test was used to compare the concentration of heavy metals between the domestic and imported retail catfish fillets. A chi-squared test was applied to check the significant difference of veterinary drug residues between the two groups. Statistical significance was accepted when $p < 0.05$. The statistical analysis was performed using R statistical software version 3.6.2 (R Development Core Team, 2019).

3. Results

3.1. Genetic authentication and mislabeling

Out of 793 "unknown" subsamples (~226 samples) received for the test, 695 samples were successfully barcoded (87.6% success rate). The successfully barcoded samples had a minimum Phred Q20 barcode length of 100 bp and a BOLD matching percentage of 99.9%. Overall quality metrics were an average Q20 barcode length of ~509 bp and an average BOLD matching percentage of ~99.9%. NJ trees and additional internal and NCBI sequences were also queried to verify that BOLD matches were accurately labeled in that system. Repeating the sequencing cycle for samples failed in the first run; those with a barcode length less than 100bp (n = 57, 7.2% of samples) or contaminated with a heavy load of bacterial colonies (n = 41, 5.2% of samples), increased the success rate of molecular barcoding to 94.2%. Among the samples failed in the first sequencing cycle, 11 samples identified in the second run. However, 46 samples remained unknown at the end. Approximately 89% of the samples were identified as channel catfish (*Ictalurus punctatus*, 65.2%) and striped catfish (*Pangasianodon hypophthalmus*, 23.8%) (Table 2).

As seen in Table 3, channel catfish and striped catfish were labeled as "catfish" and "swai" (or "tra") in the retail catfish fillets, respectively. No samples were genetically identified as *Pangasius bocourti*, known as "basa" for imported catfish in the United States. The mislabeling rate of the fillets commercially labeled as *P. hypophthalmus* was higher than those marked as *I. punctatus*. The overall mislabeling rate in this study

Table 2
Results of DNA barcoding of retail catfish fillets in the Northeast US markets.

Number of sample	Number of barcoded	Success rate (%)	Bacterial contamination (%)	*I. punctatus* number (%)	*P. hypophthalmus* number (%)
793	747	94.2	5.2%	517 (65.2%)	189 (23.8%)

Table 3
Genetic authentication of retail catfish fillets in the Northeast US markets.

		Commercial label			
	Species (Market's name)	*I. punctatus* (catfish)	*P. hypophthalmus* (swai or tra)	*P. bocourti* (basa)	Total number
Genetic confirmation	*I. punctatus*	499	18	0	517
	P. hypophthalmus	11	165	13	189
	P. bocourti	0	0	0	0
	No Identification	25	21	0	46
	Bacterial contamination	10	30	1	41
	Total number	545	234	14	793
	Mislabeling rate (%)	6.6	16.7	100	11.1

was estimated at 11.1% (Table 3).

The majority of the mislabeled samples were assigned to Cambodia (~46%, Table 4), while the minimum mislabeling rate was calculated for imported catfish fillets from China (~4%). Around 7% of domestic catfish fillets were labeled with the wrong species name. The value for the imported sample was 17.6% (Table 4).

3.2. Presence of CAP and MG/GV compounds

Out of 362 samples tested, 11 samples were detected presumptively positive with CAP (containing of 4 domestic, 5 imported from Vietnam, and 2 samples which had unknown country of origin; Fig. 1A). No significant difference (p-value = 0.303) was found between the domestic and imported samples based on number of samples tested for CAP presence. While all the domestic catfish detected presumptively positive with CAP were genetically identified as *I. punctatus*, three of the imported samples were mislabeled, labeled as basa but identified as *P. hypophthalmus*. Similarly, both samples with unknown country of origin were labeled as basa but genetically identified as *I. punctatus* and *P. hypophthalmus*.

Among all the samples, 80 samples were presumptively positive with MG/GV; including 43 domestic, 27 imported from Vietnam (n = 12), China (n = 5), Cambodia (n = 5), Thailand (n = 5), and 10 samples assigned to no country. There was no significant difference (p-value = 0.889) between the number of domestic and imported samples were presumptively positive with MG/GV (Fig. 1B). Three presumptively positive domestic samples were labeled wrongly (two samples not identified genetically and one sample was identified as *P. hypophthalmus*). The value was 4 for both imported samples and samples with not undefined country of origin which were labeled as swai or basa.

3.3. Cd, Pb, As, and Hg concentrations

The minimum detection levels (MDL) used by the USDA-FSIS for the heavy metals are as follows: Mercury – 200 μg kg^{-1}, Arsenic – 200 μg kg^{-1}, Lead – 25 μg kg^{-1}, and Cadmium – 10 μg kg^{-1}. Only one sample (domestic) had a detectable level of Hg (430.5 μg kg^{-1}). Concentration of As was not detectable in any samples.

Among samples tested for Pb, no detectable level of Pb was measured in imported sample. In contrast, one domestic sample (Pb concentration = 54.95 μg kg^{-1}) and three samples with unknown country of origin (Pb concentration = 281, 59, and 35 μg kg^{-1}) had the concentration of Pb higher than detection levels (Fig. 2). Although all the three samples were labeled as swai, one of the samples was not genetically confirmed. Likewise, the genetic survey revealed that the imported sample was *P. hypophthalmus*.

Also, 18 domestic, 15 imported samples, and 11 samples with unknown country of origin had detectable level of Cd (Fig. 3A). The imported sample were imported from Vietnam (n = 11), Thailand (n = 2), China (n = 1), and Cambodia (n = 1). They are mostly labeled as swai and basa, however, six of them were not genetically confirmed. No significant difference was detected between the number of samples with detectable level of Cd based on being domestic or imported (p-value = 0.345).

Cd concentration varied from 10.09 μg kg^{-1} to 65.92 μg kg^{-1} in the imported group of samples and it was measured from 10.21 μg kg^{-1} to 395 μg kg^{-1} in domestic catfish fillets. The range for those samples with unknown country of origin was from 10.61 μg kg^{-1} to 66 μg kg^{-1}. The concentration of Cd was not statistically different between domestic and imported products (p-value = 0.382, Fig. 3B).

4. Discussion

4.1. Genetic confirmation and mislabeling

In our study, the retail catfish fillets were successfully identified using DNA barcoding with a high success rate of 94.2%. Many studies have confirmed the method is potent at identifying species from fresh fish or seafood products (Appllewhite et al., 2016; Cawthorn et al., 2015;

Table 4
Number of mislabeled retail catfish fillets based on labeled country of origin in the Northeast US markets.

		Commercial label				
	Species (market's name)	*I. punctatus* (catfish)	*P. hypophthalmus* (swai or tra)	*P. bocourti* (basa)	Total number	Mislabeling rate (%)
Country of origin on label	USA	30	5	0	35 (495)	7.1
	China	1	0	1	2 (49)	4.1
	Cambodia	0	5	0	5 (11)	45.5
	Thailand	0	0	1	1 (6)	16.7
	Vietnam	5	29	9	43 (223)	19.3
	Not known	0	0	2	2 (9)	22.2

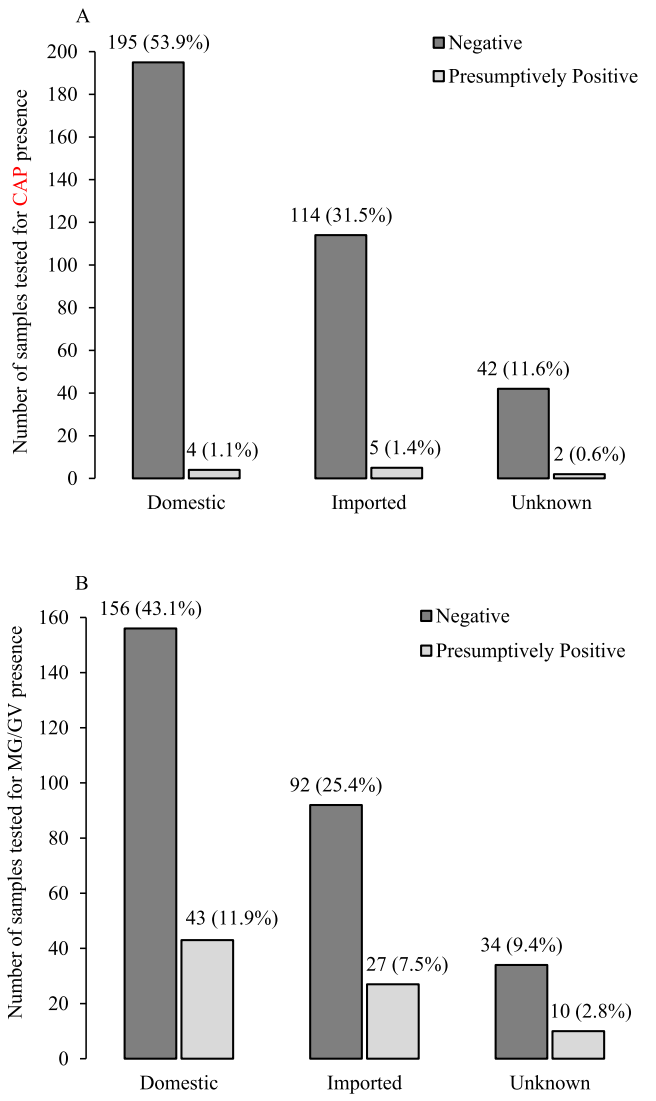

Fig. 1. Occurrence of CAP (A) and MG/GV (B) in retail catfish fillets in the Northeast US markets.

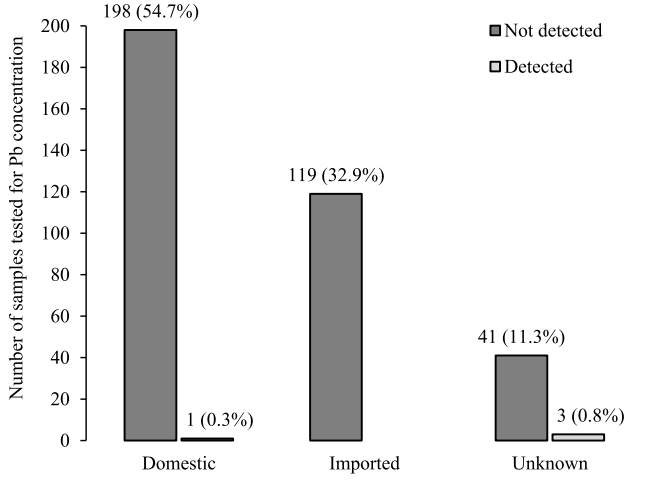

Fig. 2. Occurrence of Pb in retail catfish fillets in the Northeast US markets.

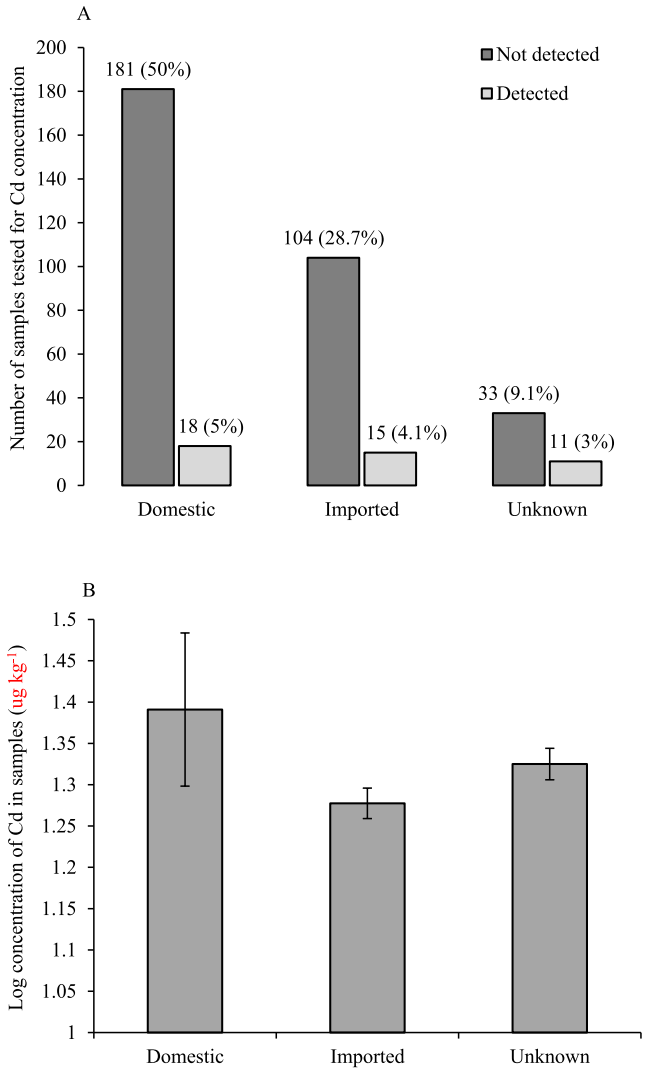

Fig. 3. Occurrence of Cd in retail catfish fillets in the Northeast US markets (A) and mean concentration of Cd in the samples which had detectable level of Cd (B). Error bars denote the standard deviations of data in the fillets with detectable levels of Cd.

Christiansen et al., 2018; Chapela et al., 2007; Hellberg & Morrissey, 2011; Tuuli et al., 2016; Willette et al., 2017). DNA barcoding accounted for 100% successfully sequenced salted or smoked fish, 92.2% cephalopods, 83.3% products mixed with cephalopods and crustaceans, 79% canned fish, 77.4% crustaceans, and 60% bivalves on imported fishery products at the Border Inspection Post of Livorno-Pisa Italy (Guardone et al., 2017). Isaacs and Hellberg (2020) combined DNA barcoding and real-time PCR in the retail fillets of red snapper *Lutjanus campechanus* and suggested the method as an efficient rapid screening tool that could be used on-site to determine the authenticity of red snapper fillets. Thus, our study, in line with previous studies, supports the use of DNA barcoding as a complementary tool for species identification and delineation.

Two major factors have been associated with the success rate of DNA barcoding: the distinctiveness of the sequence, and the extent of coverage and accuracy of the database that is used for species identification (Hajibabaei et al., 2007; Ward et al., 2009). In particular to the first factor, the mini-barcoding helps to successfully amplify fish samples that failed previously (Liou et al., 2020). Chin et al. (2016), employed mini-barcoding and full barcoding simultaneously for seafood products'

samples with different levels of food processing and achieved ~81% power of discrimination. In red snapper fillets, the barcoding success rate increased from ~81% (full barcoding) to more than 99% with the aid of mini-barcoding (Isaacs & Hellberg, 2020). Likewise, substantial improvement in the success rate, from 20.5% full barcoding to 88.6% mini barcoding, was observed by Shokralla et al. (2015). In our study, repeating the sequence cycle for mini-barcoded samples and bacterially contaminated specimens led to a slight increase in the barcoding success rate from 87.6% to 94.2% and fish species identification from 87.6% to 89%.

DNA barcoding failure depends on several reasons in a seafood sample. Intense food processes involving heating, high pressure, and sterilization extremely degrades DNA into short fragments (Cawthorn, Steinman, & Witthuhn, 2012; Hellberg & Morrissey, 2011; Lin & Hwang, 2007; Teletchea et al., 2005). Gelatinous protein also binds to nucleic acids and hinders DNA extraction (Winnepenninckx et al., 1993). In addition to the food processing level, some additive compounds such as salt and monosodium glutamate can eliminate the DNA genome through manufacturing (Kakihara et al., 2006; Meusnier et al., 2008). In our study, about 5.2% of genetically tested samples failed the sequencing process due to a heavy load of bacterial contamination as a result of different handling techniques and collection strategies. Many were from fillets shipped frozen and subsequently thawed for retail sale. Similarly, short sequences were mostly obtained from samples that were high in fat, thawed upon arrival, discolored, and/or had a foul-smelling odor. According to (Holmes et al., 2009), a high load of bacterial population can negatively target both amplification success and identification accuracy.

In the present study, DNA barcoding unveiled different ratios of substitution in catfish fillets which genetically confirmed it belongs to other species rather than *I. punctatus* and *P. hypophthalmus*. Most of the mislabeled *P. bocourti* was identified as *P. hypophthalmus*. These species can be differentiated morphologically by color, shape of snout, barbells, shape of the caudal fin, and number of anal soft rays (Kottelat, 2001; Rainboth, 1996; Yamamoto & Tagawa, 2000). Yet, they can be replaced by mistake. Therefore, they might co-mingle at US processing plants or seafood distribution facilities. Also, there is another explanation for switching *P. hypophthalmus* with *I. punctatus*. Pangasius fish fillets are low-priced products (US$4.99/Ib for "swai" in comparison to US $8.99/Ib for "catfish"). This has led to a high demand of *Pangasius* fish fillets around the world, especially in the US (FAO, 2016). On the contrary, the US Congress legislated a law so that only the Ictaluridae family must be labeled with the name of "catfish" (Brambilla et al., 2012; Duc, 2010). The combination of these facts has resulted in a high mislabeling rate among catfish products (Bosko et al., 2018; Wang & Hsieh, 2016). Since the presence of *P. bocourti* is not genetically confirmed in our study, additional analysis is needed to determine whether any of the barcoded samples were sold under the name *basa* and how commonly the *basa* label is applied in the US retail markets. Pappalardo and Ferrito (2015) suggested that the substitution of European plaice (*Pleuronectes platessa*) with the common sole (*Solea solea*) could have happened by accident as they could have been switched before being filleted.

The countries involved in mislabeling the most were Cambodia, Vietnam, and Thailand. The high rates from these countries are not surprising considering *P. hypophthalmus* and *P. bocourti* mainly are aquaculture in South Asia and Southeast Asia including Vietnam (Delaware Sea Grant, 2017). Besides, Vietnam exported these species with the commercial label of "catfish" initially (Duc, 2010). Guardone et al. (2017) named China, Vietnam, and Thailand as having the most contribution in mislabeling. According to the Rapid Alert System for Food and Feed (RASFF), Thailand, Vietnam, China, Ghana, and Senegal ranked the top countries regarding the number of mislabeling cases (RASFF Portal, 2016). Lack of a legislation system on seafood traceability and an official naming system was claimed to be the main reason for the observed pattern in these countries (Xiong et al., 2016).

4.2. Veterinary drugs and heavy metal contaminants

Except for *Pseudomonas* spp., CAP is an effective antibiotic against both gram-positive and gram-negative bacteria (Abdollahi & Mostafalou, 2014). It also showed prohibitive activity against a broad spectrum of other microorganisms (Samanidou & Evaggelopoulou, 2007). The antibiotic has been banned for use in food animals on account of its harmful effects to public health such as carcinogenicity, bone marrow depression, and fatal aplastic anemia (Chukwuka Okocha et al., 2018; FDA, 2017; Rupp et al., 2011). According to the FDA (2002), the maximum residue limit for CAP is zero. Several reports have been confirmed using antibiotics in aquaculture to support intensive fish farming and to save on the cost of feed (Defoirdt et al., 2007, 2011; Rico et al., 2013), especially CAP (Hassan et al., 2013; Lu et al., 2009). In the US, the rate of veterinary drug violation in seafood products was reported 24 detections per year on average (Love et al., 2011, pp. 7232–7240). The violation occurrence in finfish was ascribed mostly to catfish or fish sold as catfish and the highest rate was detected in the products farmed in Vietnam and China followed by Indonesia, United States, Thailand, not indicated, India, and Taiwan (Love et al., 2011, pp. 7232–7240). However, the CAP was not detected in the fish samples (Love et al., 2011, pp. 7232–7240). In our study, 11 catfish fillets (4 domestic, 5 imported from Vietnam, and 2 Unknown origin) were detected presumptively positive with CAP (~3%), indicating possible misuse of the antibiotic or potential contamination risk during the processing and packaging in aquaculture. The occurrence of presumptively positive samples was higher in comparison to the previous findings of Ozbay et al. (2013) in which none of the retail catfish nuggets were detected presumptive positive. Considering that the percentage of presumptive positive samples was slightly higher in imported catfish fillets, suggest that violation of the US veterinary drug residual standards has occurred.

Malachite green is a well-known compound used against fungal and parasitic infections (Liang et al., 2006). Similar to CAP, malachite green compounds (MG and LMG) are classified as zero-tolerance drugs meaning that no residue is permitted to be detected in food or animal feed (Heberer, 2009, 2011; Rahkonen & Koski, 2002). Apart from its potential genotoxic and carcinogenic effects to human health (Donya et al., 2012), malachite green is commonly used in aquaculture for the sake of meeting maximum production capacity and preventing fish disease outbreak (Shariff et al., 2000). According to Love et al. (2011, pp. 7232–7240), malachite green was the most common drug residue accountable for finfish drug residue violation (77%), with an average concentration of 10.9 μg kg^{-1} (95% CI = 6.31–18.7 μg kg^{-1}) and crystal violet only comprised 6% of violation, with an average concentration of 27 μg kg^{-1} (95% CI = 4.1–176.1 μg kg^{-1}). After being absorbed by fish, malachite green and crystal violet are rapidly metabolized and reduced to their persistent leco-form metabolites (Thompson et al., 1999). Therefore, a multi-residue detection method including an oxidative step is needed to convert the leco-types to their parental form (Andersen et al., 2009) in order to measure the compounds simultaneously (Dubreil et al., 2019; Park et al., 2020, pp. 109–117). However, the method is difficult to develop due to the fact that the material used in the dye analysis or some of the compounds undergo decomposition during the oxidation process (Dubreil et al., 2019). For this reason, the method is proposed as a surveillance method and a routine demonstration on positive samples is suggested before implementation (Dubreil et al., 2019). In the present study, a substantial proportion of samples (~22%) were detected presumptively positive with MG/GV residue which may be corroborating evidence of practicing MG in the US and the countries that the fillets are imported from. Similar results have been reported by Bajic et al. (2007), Tripathi et al. (2007), Bilandzic et al. (2012), WuSheng et al. (2013), and Kwan et al. (2018).

Although, enzyme-linked immunosorbent assay (ELISA) is one of the standard reference methods for the screening drug residues in aquatic products (Impens et al., 2003), it involves a complicated pretreatment

(Zhou et al., 2014) which the method's accuracy depends on. Many interfering factors can cause biased ELISA results, for example, high concentration of endogenous compounds may mask the presence of the target veterinary drug or contamination of samples may lead to false positive errors (Johnson, 2014). To address the uncertainty, further confirmation of the presumptive positive samples has been suggested in many studies (Impens et al., 2003; Johnson, 2014; Yibar et al., 2011). In our study, the samples were only screened for the presence of CAP and MG/GV presence. Therefore, the results should be interpreted with caution. Presumptive positive samples were sent to the USDA ARS Athens, GA for further testing and overall safety of Siluriformes family can be found in the report published by USDA-FSIS (2015).

It has been reported that heavy metal contaminants have long-lasting toxic effects (Sthanadar et al., 2015; Ukoha et al., 2014) and can pose human health risks when accumulated through the food chain beyond the acceptable limits (Rauf & Javed, 2007). No samples had a detectable concentration of As in our study, representing no hazard for public health. Also, no imported sample had detected level of Hg, assuring that imported catfish fillets meet the US food safety regulation in terms of Hg concentration. However, our study detected high levels of Hg in one domestic *Pangasius* fillet. Previous studies have been reporting contradictory observations about As and Hg concentration in the imported fish fillets. For example, while *Pangasius* fillets imported from Vietnam to Brazil had no detectable concentration of As in the study of Molognoni et al. (2016), the high level of Hg was detected in *Pangasius* fillets imported from Vietnam to Italy (Ferrantelli et al., 2012). This dissimilarity demonstrates that the accumulation of these metals varies with the habitat of fish, and the farm in which they come from (Phan et al., 2013).

The occurrence of samples with detectable levels of Pb and Cd was more frequent in our study. However, the concentrations were lower than the maximum permissible levels recommended by FAO/WHO (2002). Since fish can uptake heavy metals from both water and food (Jiang et al., 2014), there is a considerable variation between concentrations of heavy metals in fish cultured under different fish farm practices. Reham (2012) screened heavy metal residue in the imported *P. hypophthalmus* fish fillets and reported that the majority of tested fish exceeded the permissible limits for Hg and Pb. According to Baki et al. (2018), As was detected in no fish products and the hierarchy of heavy metals in the fish samples was Fe > Cd > Zn > Pb > Cu > Cr > Mn > Hg. Santerre et al. (2001) reported that Cd and Pb were detected in 2% and 11% of farmed catfish (*I. punctatus*) respectively and the mean concentration of Pb was higher than Cd. Conversely, concentrations of Pb and Cd were not detectable in the retail catfish fillets (*P. hypothalamus*) originally imported from Vietnam and purchased in Gaza City (Elnabris et al., 2013). Heavy metal concentration and detection of veterinary drug residues in our study highlighted the essence of a continuous monitoring program for controlling the food safety of the retail catfish in the Northeast US markets.

5. Conclusions

Our results demonstrate the reliability of DNA barcoding (success rate of ~94%) as a method for the identification of unknown seafood products. DNA barcoding also revealed species mislabeling in ~11% of all samples; including ~18% of imported and ~7% domestic fillets. The results highlight the urgency for monitoring the seafood mislabeling program using a rapid and precise method. Provided that a substantial number of mislabeling fillets were not assigned to a specific country (unknown country of origin, ~22% mislabeling), stringent regulations should be considered to assure the accuracy of the details cited on labels. In our study, the present of CAP and MG/GV were detected presumptively positive in ~3% and ~22% of samples. The presumptively positive samples may indicate misuse of these prohibited veterinary drugs in aquaculture. Aquaculture owners should be informed of their hazards to public health if they use those chemicals. Both domestic and imported catfish fillets in our study meet the US food safety regulation in terms of As and Hg concentrations. However, the number of samples which had detectable levels of Pb and Cd was frequent, as it was measured in ~1% and ~12% of total samples for the detectable concentration of Pb and Cd, respectively. This signifies that the national monitoring program should be intensified to control heavy metal concentrations and achieve permissible levels. Therefore, a combination of DNA barcoding techniques and monitoring the presence of veterinary drug residue and heavy metal concentrations can improve transparency in the seafood market, decrease the rejection of shiploads by foreign buyers in the export sector, and guarantee food safety.

Data availability

Data generated in this article is available upon request from the corresponding author at gozbay@desu.edu.

CRediT authorship contribution statement

Gulnihal Ozbay: Supervision, analyses and drug residues testing of catfish samples, conducted screening along with the research reports to the funding agencies. **Balaji Kubandra Babu:** Funding acquisition, and prepared this manuscript together, analyzed the catfish samples for drug residues and heavy metals and assisted. **Eric Peatman:** led the DNA barcoding and genetic identification monitoring, preparation of initial report to the funding agency on the genetic confirmation results, and revision and editing of the manuscript. **Zohreh Mazaheri Kouhanastani:** analyzed the catfish samples for drug residues and heavy metals and assisted, assisted with the statistical analysis, Writing – original draft, and data analysis for this project.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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