Food Control 53 (2015) 81–90

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

# A handheld sensor assay for the identification of grouper as a safeguard against seafood mislabeling fraud

Robert M. Ulrich [a, *], David E. John [b], Geran W. Barton [c], Gary S. Hendrick [a], David P. Fries [c], John H. Paul [a]

[a] College of Marine Science, University of South Florida, 140 Seventh Ave. S., St. Petersburg, FL, 33701, USA
[b] Department of Biological Sciences, University of South Florida St. Petersburg, 140 Seventh Ave. S., St. Petersburg, FL, 33701, USA
[c] College of The Arts, University of South Florida, 4202 E. Fowler Ave., Tampa, FL, 33620, USA

ARTICLE INFO

*Article history:*
Received 11 August 2014
Received in revised form
9 January 2015
Accepted 10 January 2015
Available online 28 January 2015

*Keywords:*
RT-NASBA
FDA seafood List
Grouper
Mislabeling

ABSTRACT

Increases in international trade and global seafood consumption, along with fluctuations in the supply of different seafood species, have resulted in fraudulent product mislabeling. Grouper species, due to their high demand and varied commercial availability, are common targets for mislabeling by exploiting inefficient inspection practices. Compounding this problem is the fact that there are currently 64 species of fish from eleven different genera allowed to be labeled "grouper" per U.S. Food and Drug Administration guidelines. This wide diversity makes it difficult for regulators to discern legally salable groupers from restricted species. To obviate taxonomic misidentification when relying on external phenotypic characteristics, regulatory agencies are now employing genetic authentication methods which typically offer species-level resolution. However, standard genetic methods such as DNA barcoding require technical expertise and long turnover times, and the required instrumentation is not amenable for on-site analysis of seafood. To obviate some of these limitations, we have developed a handheld genetic sensor that employs a real-time nucleic acid sequence-based amplification assay (RT-NASBA) previously devised in our lab, for the analysis of fish tissue in the field. The base RT-NASBA assay was validated using a lab-based, benchtop RNA purification method as well as non-portable, commercial RT-NASBA analyzer. Described herein, is an uncomplicated method for purifying RNA from fish tissue in the field, which had similar efficiency to the benchtop method demonstrated through direct comparisons. We have also demonstrated that the field sensor is only slightly less sensitive than the benchtop instrument, and could discern 80.3% of groupers (no target sequence available for three species) on the 2014 FDA Seafood List from potential impostors. The complete field assay requires fewer than 80 min for completion and can be performed outside of the lab in its entirety.

Published by Elsevier Ltd.

## 1. Introduction

Grouper are the third-most economically valuable seafood product in the state of Florida, with a dockside value estimated at over $24 million (Putnam, 2012). Due to supply limits caused by increased regulation on commercial quotas, as well as their high market value, grouper are often targets for fraudulent mislabeling. Presently, there are 64 finfish species in the family Serranidae spanning eleven separate genera that can carry the acceptable market name "grouper" according to the U.S. Food and Drug Administration (FDA) in conjunction with the National Marine Fisheries Service (FDA, 2014a, 2014b). The majority of these species are included in three genera: *Epinephelus* (37 species), *Cephalopholis* (8 species), and *Mycteroperca* (9 species). The remaining species are included in 8 minor genera: *Aethaloperca* (1 species), *Anyperodon* (1 species), *Caprodon* (1 species), *Dermatolepis* (1 species), *Diplectrum* (1 species), *Plectropomus* (2 species), *Variola* (1 species), and *Hyporthodus* (2 species). To further complicate grouper identification, there have been some recent suggested taxonomic revisions to the monophyletic classification of Serranidae as well as reclassification of some members of the *Cephalopholis*, *Epinephelus*, *Hyporthodus*, and *Mycteroperca* genera (Craig & Hastings, 2007).

* Corresponding author. College of Marine Science, MSL 119, University of South Florida, 140 Seventh Ave. South, St. Petersburg, FL 33701, USA. Tel.: +1 727 553 1647; fax: +1 727 553 1189.
*E-mail address:* rmulrich@mail.usf.edu (R.M. Ulrich).

http://dx.doi.org/10.1016/j.foodcont.2015.01.022
0956-7135/Published by Elsevier Ltd.

However, the FDA has yet to fully recognize these amendments. Due to the diversity of grouper species, accurate taxonomic identification can be difficult, particularly when the head and skin are removed to market them as fillets (Buck, 2010).

Oceana recently reported findings from one of the largest seafood fraud investigations in the world, performed over a two-year period to determine the prevalence of mislabeled fish sold by seafood retailers in the U.S. DNA analysis of over 1200 seafood samples from 21 states revealed that approximately one-third were mislabeled with regard to FDA legal trade criteria (Warner, Timme, Lowell, & Hirshfield, 2013). Grouper were chosen as one of the finfish categories of interest and 26% were found to be mislabeled farmed Asian catfish (*Pangasius* spp.), freshwater perch, weakfish, bream, or king mackerel. Asian catfish imported into the U.S. are primarily farmed in Vietnam along the Mekong River, a body of water that has become polluted in many areas due to increases in unregulated mining activity and anthropogenic run-off (Fu et al., 2012; Ongley, 2009). Moreover, Vietnamese fish farming regulations are often less stringent than those of domestic aquaculture. Antimicrobial compounds such as malachite green and enrofloxacin (Baytril®) have been found in imported *Pangasius* catfish fillets, both of which are prohibited from use in U.S. food production per FDA criteria (Staff, 2011). Also disturbing was the discovery of king mackerel surrogate fillets which often contain high levels of mercury to the extent that the FDA advises against consumption by sensitive groups such as children and pregnant women (FDA, 2004).

The Oceana investigation also revealed that at least one fish sample, mislabeled as legally salable grouper, was actually Speckled Hind (*Epinephelus drummondhayi*) which is a species on the International Union for Conservation of Nature and Natural Resources (IUNC) Red List as critically endangered (Chuen & Huntsman, 2013). This type of seafood fraud undermines conservation efforts put in place to prevent overfishing of at-risk species by making illegal fishing profitable. Furthermore, mislabeling can negatively affect estimates of fish stocks used in fisheries management by contradicting the true state of the fishery. The misidentification of species before commercial landings data are reported to regulatory agencies can cause a two-fold consequence by both inflating fisheries-dependent landings data of the more desired species as well as underestimating catches of less desirable, and possibly unmanaged species (Jacquet & Pauly, 2008; Marko et al., 2004).

In 2009, the South Atlantic and Gulf of Mexico Region's seafood industry generated approximately $13 billion in sales impacts and created 65,000 jobs for Florida, of which grouper accounted for 10 percent of all non-shellfish total landings revenue (NMFS, 2010). The demand for grouper in the U.S. is so strong that it cannot be met by the harvesting of domestic species alone. In 2012, over four thousand metric tons of foreign grouper, worth approximately $33.5 million, were imported into the U.S. (NOAA, 2012). This mass quantity of imported grouper creates numerous foreign outlets for the sale of fraudulently mislabeled fish. Recently, a group of U.S. seafood wholesalers were convicted of felony offenses for their roles in purchasing and selling farm-raised Asian catfish which were purposely mislabeled as grouper (Staff, 2011). In addition to misleading consumers into paying more for lesser-valued seafood species, fraudulent mislabeling negatively affects consumer confidence in domestic seafood, which can undercut the profits of local grouper fishermen (Ropicki, Larkin, & Adams, 2010). Seafood fraud is also detrimental to the national economy, as some importers may mislabel to avoid paying tariffs and anti-dumping duties. In 2010, Vietnamese catfish were sold as grouper to evade over $63 million in tariffs that would have been owed to the U.S. government (FDA, 2010).

As of 2011, the sole standard method used by the FDA for forensic identification of seafood species was isoelectric focusing (IEF) on soluble muscle proteins, which is meant to produce species-specific electrophoretic banding patterns when referenced against a database consisting of vouchered specimens (AOAC, 1980). While this method has proved somewhat dependable when testing unprocessed seafood under cold storage, it has been described as unreliable when analyzing cooked tissues or fillets packaged with acidic sauces or marinades to increase value (Applewhite, Rasmussen, & Morrissey, 2012; Rasmussen & Morrissey, 2008). Furthermore, IEF procedures are tedious, often require several days, and interpretation of results can be subjective (Applewhite et al., 2012). To obviate some limitations of IEF, the FDA has recognized the need to transition towards DNA-based forensic analysis of seafood. Additionally, genetic analysis may offer higher species-level resolution than protein-based assays due to the variable degeneracy of some nucleotide sequences that are not translated into proteins between some closely related species (Rasmussen & Morrissey, 2008).

The FDA recently approved DNA barcoding as an acceptable method for seafood species identification (Hanner, 2011). This method involves the amplification of a specific gene locus by PCR, primarily the cytochrome *c* oxidase subunit I gene (COI or COX1), and performing nucleotide sequencing reactions on the amplicons. The unknown sequence is then referenced to a database consisting of sequence submissions generated from verified specimens of respective species (Handy et al., 2011; Yancy et al., 2008). The particular database utilized by the FDA is a growing compilation of barcodes gained from their own sequencing efforts, using only authenticated and vouchered specimens as source tissue (http://www.fda.gov/Food/FoodScience//ucm238880.htm). Barcoding is advantageous in that it relies on validated standard methods and gene loci; however, the requirement of DNA sequencing machinery obligates the outsourcing of analysis to a select number of labs having this capacity which creates prolonged turnover times.

There is an emerging interest in genetic identification technologies for seafood that reduce processing time and eliminate the need for lab-based DNA sequencing. An alternate technology termed TwistFlow® Red Snapper (http://www.twistdx.co.uk) utilizes recombinase polymerase amplification (RPA), which allows for the field identification of red snapper (*Lutjanus campechanus*) in conjunction with a portable real-time fluorometer. Due to the isothermal nature of RPA, conventional PCR thermal cycling is not required freeing the system from the necessity of large lab-based thermal cyclers with high power demands, making it amenable to field applications.

We have previously developed a real-time nucleic acid sequence-based amplification assay (RT-NASBA) that is able to differentiate most of the FDA allowable groupers from potential surrogate fish species by targeting a region of the mitochondrial 16S rDNA gene (Ulrich et al., 2013). NASBA is an isothermal RNA amplification method that, when used in conjunction with fluorescently-labeled molecular beacons, provides real-time identification of specific nucleotide sequences (Compton, 1991; Tyagi & Kramer, 1996). The prior validation of this assay was performed using a commercial lab benchtop NASBA analyzer (bioMérieux, NucliSENS EasyQ®) which cannot be easily transported for on-site forensic analysis of fish tissues. Here we report the development of a portable grouper forensics test by integrating our RT-NASBA assay with an in-house designed handheld heated fluorometer dubbed QuadPyre, which is a refined evolution of a prototype sensor developed by our group (Casper et al., 2007). We have also devised a simple and inexpensive method for the field purification of RNA from fish tissue allowing the entire analysis to be performed in the field in less than 80 min. We believe this technology will provide a useful on-site screening tool to aid seafood processors, distributors, retailers and restaurateurs in remaining compliant with compulsory FDA regulations on salable grouper species.