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# DNA barcoding reveals a high incidence of fish species misrepresentation and substitution on the South African market

Donna-Mareè Cawthorn [a,*], Harris Andrew Steinman [b], R. Corli Witthuhn [a]

[a] Department of Food Science, University of Stellenbosch, Private Bag X1, Matieland, 7602, South Africa
[b] Food & Allergy Consulting & Testing Services (F.A.C.T.S), P.O. Box 565, Milnerton, 7435, South Africa

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ABSTRACT

The mislabelling of fishery products has emerged as a serious problem on global markets, raising the need for the development of analytical tools for species authentication. DNA barcoding, based on the sequencing of a standardised region of the cytochrome *c* oxidase I (COI) gene, has received considerable attention as an accurate and broadly applicable tool for animal species identifications. The aim of this study was to investigate the utility of DNA barcoding for the identification of a variety of commercial fish in South Africa and, in so doing, to estimate the prevalence of species substitution and fraud prevailing on this market. A *ca.* 650 base pair (bp) region of the COI gene was sequenced from 248 fish samples collected from seafood wholesalers and retail outlets in South Africa, following which species identifications were made in the Barcode of Life Database (BOLD) and in GenBank. DNA barcoding was able to provide unambiguous species-level identifications for 235 of 248 (95%) samples analysed. Overall, 10 of 108 (9%) samples from wholesalers and 43 of 140 (31%) from retailers were identified as different species to the ones indicated at the point of sale. Although some cases of mislabelling were potentially unintentional due to misapplied market nomenclature, a far greater proportion represented serious and seemingly deliberate acts of fraud for the sake of increased profits. This study has highlighted that the existing legislation pertaining to seafood marketing in South Africa is inadequate or poorly enforced and requires urgent revision. In the light of the results presented here, DNA barcoding appears to hold great potential for fish authentication monitoring by both regulatory bodies and industry, the utilisation of which could enhance transparency and fair trade on the domestic fisheries market.



## 1. Introduction

The world's marine fish stocks, which were considered just over a century ago to be 'inexhaustible' (Huxley, 2007), now face extreme fishing pressure as the insatiable human appetite for seafood continually outpaces supply (Delgado, Wada, Rosegrant, Meijer, & Ahmed, 2003). Current data indicate that widespread overfishing has fully exploited, over-exploited or depleted up to 75% of global fish stocks (FAO, 2009) and has had deleterious effects on aquatic ecosystems (Pauly, Watson, & Alder, 2005; Worm, Barbier, & Beaumont, 2006). In a pertinent four-year study on 10 large marine ecosystems around the world, Worm, Hilborn, Baum, et al. (2009) reported that 63% of the assessed fish stocks were below desired levels and still require rebuilding, in spite of the numerous restrictions (annual harvest quotas, rights allocations, fishing gear modifications and seasonal or area closures) that have been imposed to promote more sustainable fisheries management (Beddington, Agnew, & Clark, 2007; Brunner, Jones, Friel, & Bartley, 2009).

During the last two decades, there has been a growing realisation that the incorporation of consumer behaviour into marine conservation strategies will be required if the trends in fisheries declines are to be reversed (Kaiser & Edward-Jones, 2006). This realisation has led to a number of sustainable seafood awareness campaigns being initiated in many parts of the world, including the United Kingdom (UK), United States (US), Australia and Canada. The Southern African Sustainable Seafood Initiative (SASSI) was established in 2004 with similar aims of educating the local population on marine conservation issues and shifting consumer choices towards more sustainable seafood species. Typically, such organisations compile seafood lists that rank species according to sustainability criteria (e.g. 'best choice' or 'avoid'), the details of which are publicly disseminated via wallet cards, electronic databases and mobile phone applications (Roheim & Sutinen, 2006). A fundamental requirement for the success of all consumer awareness campaigns, as well as for fisheries management in general, is the accurate naming and labelling of fish products at the point of sale. Unfortunately, with escalating demand and globalisation of seafood trade, the current market climate in many countries

* Corresponding author. Tel.: +27 21 808 3578; fax: +27 21 808 3510.
*E-mail address:* 13946455@sun.ac.za (D.-M. Cawthorn).

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is highly conducive to fraud and mislabelling of fish products (Jacquet & Pauly, 2007).

The mislabelling of fish species can manifest in several forms, as well as at any stage in the fisheries supply chain (Logan, Alter, Haupt, Tomalty, & Palumbi, 2008). A portion of the mislabelling that occurs is likely unintentional as fish species identities may be easily mistaken. Confusion may also arise due to the fact that different fish species can be referred to by a common vernacular name, or by different vernacular names in different regions (Buck, 2009). Of greater concern, however, is that some unscrupulous traders may deliberately use mislabelling as a means to launder illegally-caught fish into legitimate markets, or simply to defraud consumers for the purpose of accruing greater profits (Ogden, 2008). Since the flesh of many fish species is similar in appearance, taste and texture, it becomes relatively easy for species of high commercial value to be substituted, either partially or entirely, with species of lower value. The lack of traceability in the fisheries supply chain also provides a considerable opportunity for mislabelling. Fish products often change hands several times on route from the fishing vessels to the consumer's plate, making it difficult to identify the link in the supply chain where the fraud or substitution occurred (Thompson, Sylvia, & Morrissey, 2005).

Whether accidental or deliberate, fish mislabelling is not only a form of economic deception, but it also undermines the efforts of seafood awareness campaigns and can further erode already threatened fisheries (Jacquet & Pauly, 2007). For instance, 77% of the fish labelled as 'red snapper' in the US have been found to be substituted with less expensive and/or overexploited species (Marko, Lee, Rice, et al., 2004). In South Africa, shortfin mako shark has been sold as 'ocean fillets' or 'sokomoro' to increase its appeal (Atkins, 2010), even though it is listed as 'vulnerable' by the International Union for Conservation of Nature (IUCN, 2010). Furthermore, just as ichthyologic name-swapping can prevent consumers from making choices in favour of conservation, it also infringes on their right to safeguard their own health. Certain fish species can cause fatal allergic reactions (Triantafyllidis, Karaiskou, Perez, et al., 2010), while others contain potent toxins or high levels of contaminants. Reports have emerged on the mislabelling of pufferfish and oilfish as 'monkfish' and 'cod', respectively, where both cases have caused serious illness (Cohen, Deeds, Wong, et al., 2009; Lam, 2007).

Government regulations in many countries, including South Africa, require the full disclosure of food product content and stipulate that food labelling must not be misleading (DoH (Department of Health, South Africa), 2010; Martinez, James, & Loréal, 2005; NRCS, 2003). Nevertheless, such provisions have done little to deter mislabelling as they are often poorly enforced, or because the penalties for non-compliance are small in comparison to the profits resulting from fraudulent fish trading (Buck, 2009). There is now mounting evidence that molecular species identification methods, particularly those based on DNA analysis, can serve as critical tools for industry self-regulation, governmental monitoring and prosecution of illegal activates (Ogden, 2008). In particular, DNA barcoding – the sequencing of an approximately 650 base pair (bp) region of the cytochrome *c* oxidase I (COI) gene – has gained widespread support in the scientific literature as a rapid, cost effective and standardised method for the identification of a diverse range of animal lineages, including fish species (Hebert, Cywinska, Ball, & deWaard, 2003a; Hebert, Ratnasingham, & deWaard, 2003b; Ward, Zemlak, Innes, Last, & Hebert, 2005). This mitochondrial DNA (mtDNA) locus has been validated as a diagnostic marker for forensic identification applications (Dawnay, Ogden, McEwing, Carvalho, & Thorpe, 2007). In addition, COI barcoding is under consideration by the United States Food and Drug Administration (FDA) for uptake into their current regulatory framework and to serve as a replacement for the technique of protein isoelectric focusing for fish species identification (Handy, Deeds, Ivanova, et al., 2011; Ward, Hanner, & Herbert, 2009; Yancy, Zemlak, Mason, et al., 2008). Adoption of the COI gene for DNA barcoding purposes by the Consortium for the Barcode of Life (CBOL) has led to the initiation of a number of international collaborative research efforts, including the Fish Barcode of Life Initiative (FISH-BOL), which aims to barcode all fish species of the world (Steinke & Hanner, 2011; Swartz, Mwale, & Hanner, 2008; Ward et al., 2009).

DNA barcoding has been utilised to evaluate the incidence of fish species substitutions in North America (Wong & Hanner, 2008), Europe (Miller & Mariani, 2010) and Italy (Barbuto, Galimberti, Ferri, et al., 2010; Filonzi, Chiesa, Vaghi, & Nonnis Marzano, 2010). However, to date, there have been no published reports on the use of this method to estimate the prevalence of such substitutions in South Africa. Considering that South Africa plays a leading role on the African continent in terms of both fish production and trade (INFOSA, 2007), such an evaluation is imperative to determine the incidence of mislabelling that could perpetuate locally or in exported commodities. The aim of this study was to investigate the utility of DNA barcoding for the identification of a large variety of fish products commercially traded at the wholesale and retail levels in South Africa, and in so doing, to assess the extent of misrepresentation and substitution occurring on this market.

## 2. Materials and methods

### 2.1. Sample collection

Fish samples were collected over a two-year period (2008–2010) in four provinces of South Africa, namely the Western Cape (WC), Eastern Cape (EC), KwaZulu-Natal (KZN) and Gauteng (GP). The former three provinces are the major coastal fishing provinces in South Africa and were included as these were expected to have access to a large variety of locally-caught fish species. Gauteng (GP) was included in order to evaluate the commercial fish trading practices in an inland province, principally because it is the most populated province in South Africa with the highest per capita income (Schlemmer, 1998). A total of 257 samples were collected, of which 108 (42%) were obtained from the wholesaler/distributor level, while 149 (58%) were obtained from retail outlets, which included both supermarkets and fish markets. Supermarkets were defined as those stores that sold fish and various other grocery items, while fish markets were defined as those stores selling primarily seafood commodities. All samples collected from wholesalers/distributors were purchased frozen, but these included both whole and processed specimens. Fresh, frozen, whole and processed fish samples were acquired from the retail outlets. All samples were stored in a laboratory freezer (−20 °C) following collection.

### 2.2. DNA extraction

Tissue was excised from the lateral muscle of each fish specimen with a sterile scalpel and forceps. Total genomic DNA was extracted from *ca.* 500 mg of the muscle tissue using the SureFood® PREP Allergen Kit (r-Biopharm, supplied by AEC-Amersham, Cape Town, South Africa), following the manufacturer's instructions. The concentrations and purities of the extracted DNA were assessed in a spectrophotometer (Beckman Coulter DU530, Beckman Instruments, Fullerton, USA) at 260 and 280 nm. DNA extracts were stored at −20 °C prior to further analysis.

### 2.3. Polymerase chain reaction (PCR)

A 652 base pair (bp) fragment from the 5′ region of the COI gene was PCR amplified using the M13-tailed primer cocktail (C_FishF1t1/C_FishR1t1) previously described for the DNA barcoding of fish species (Ivanova, Zemlak, Hanner, & Hebert, 2007). The 25 μl PCR reaction mixtures contained 2.5 μl (1×) reaction buffer ($MgCl_2$ free) (Super-Therm, supplied by Southern Cross Biotechnologies, Cape Town, South Africa), 2.5 μl (2.5 mM) $MgCl_2$ (25 mM, Super-Therm), 0.25 μl (100 nM) of each primer (10 μM stocks), 0.125 μl (0.625 U) *Taq* DNA polymerase