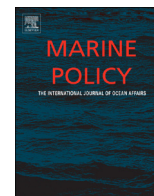




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A systematic analysis across North Atlantic countries unveils subtleties in cod product labelling



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ABSTRACT

Over the last decade, the mislabelling of seafood products has come into prominence in the fields of food science and marine conservation. This study aims to determine whether differences in fish labelling accuracy can be explained by factors associated with governance, legislation and product availability, using cod (*Gadus* spp.) as a case study. A total of 401 cod products from a range of different supermarket retailers in each of nine countries bordering the North Atlantic Ocean were purchased and genetically identified. The countries sampled were grouped into primarily cod-importing or cod-producing states, and belonging/not-belonging to the European Union. They comprised the United Kingdom, Belgium, the Netherlands, Denmark, Estonia, Iceland, Norway, Sweden and Canada. Estonia showed the highest incidence of mislabelling, with 59.4% samples mislabelled, followed by Denmark with 18.6%, Canada with 7.3%, Sweden with 4.4% and finally the United Kingdom with 2.4%. Substitute species included species within the Gadidae and Merlucciidae, such as haddock (*Melanogrammus aeglefinus*), Alaskan pollock (*Gadus chalcogrammus*) and Argentine hake (*Merluccius hubbsi*), respectively, but also included species more distantly-related to cod, such as snailfish (*Liparis* spp.), spotted wolffish (*Anarhichas minor*) and yellow perch (*Perca flavescens*), the latter a freshwater species. The remaining countries showed no mislabelling. Neither EU affiliation, production nor the type of product, i.e. fresh or processed, had a significant effect on mislabelling. It is suggested that other factors, such as country-specific differences at social, cultural or legal levels, may be the greater drivers of mislabelling.

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1. Introduction

The increase in seafood supply, international trade and progress in food processing have created the potential for species substitution, which has become a major concern both in domestic and international markets (reviewed in [1]). Products that are traded internationally are generally processed to some extent, thereby removing the morphological characteristics required for species authentication and making products vulnerable to mislabelling [2]. Furthermore, seafood supply chains are getting progressively longer and processing steps are often carried out in different countries, increasing the opportunity to mislabel food.

The United Nations Food and Agriculture Organization (FAO) *Codex alimentarius* requires the country of origin of all food products to be identified, except when food has undergone processing in another country; in this case, the country where processing took place is

considered country of origin [3]. In Europe, the principles for traceability and food safety are laid down by a plethora of regulations and directives [4–8]. These pertain to the requirements that all fish and fishery products must be traceable throughout all stages of production, processing and distribution and accurate labelling must be present on all food products, including: the commercial and scientific name of the species, the method of production (wild or farmed), and the catch area [5,8]. Furthermore, seafood must not be sold under a name that could mislead the consumer as to its true identity [4]. In contrast, in both Canada and the USA, the labels of packaged fresh seafood products are only required to include an appropriate common name, compiled in the CFIA Fish List and Food and Drug Administration (FDA) Seafood List, respectively. Additionally, in some cases, the “country of origin” may be required, however this may just be the last country in which part of the product processing has taken place [9–11]. Furthermore, the country of origin of seafood products imported into Canada must be declared on all imported fish products, but only on the container in which they are imported, not necessarily on the retail package [11].

Mislabelling is the process of substituting one species for another. There is widespread evidence of seafood mislabelling,

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otherwise known as species substitution, occurring on a range of species and geographical scales, despite the existence of seemingly adequate and specific policies relating to product traceability. This phenomenon holds implications for the conservation and management of marine resources and human health [12–14], causes economic losses [15] and harms consumer perception [16] and eco-campaigns [17].

Mislabelling can have dire consequences for overfished species or those that are under protection. Nearly 80% of smooth-hound products (“palombo”) sold in Italy did not belong to *Mustelus* spp., which are the only species permitted to be sold under this vernacular name [18]. In fact, many of the species identified are listed on the IUCN Red List, rated as vulnerable and near threatened. The US FDA Seafood List designates 13 species of rockfish that can be sold under the common name “Pacific red snapper”, however, an investigation found that > 60% of “Pacific red snapper” products contained species that were not included in the list, some of which were listed as overfished [19]. Furthermore, a study of seafood fraud in the USA by the conservation group Oceana found one in three samples to be mislabelled [20]. Market substitution appears to be consistently more conspicuous in North America [21,22] than in Europe [23,24], although recent surveys of restaurants have revealed considerably greater levels of substitution than found in the retail sector [25].

The increased use of molecular genetic markers should protect both consumers and producers from fraud and safeguard species from over-exploitation and illegal trafficking [26,27]. In recent years, DNA barcoding has emerged as a broadly applicable tool for species identification [28]. The DNA barcoding gene *cytochrome oxidase 1* (COI) has been validated as a diagnostic marker for species-level identification in birds, fish and invertebrates [29–32]. DNA barcoding makes use of an inexpensive and high throughput technology and can be used to identify whole or parts of specimens to enable the identification of species that are protected and/or harvested illegally [26,27,33]. Given the background presented above and the global importance of cod fisheries, a deeper understanding of cod products in international markets is of particular interest. This study expands on recent investigations [16,24,34,35] and assesses the prevalence of Atlantic cod (*Gadus morhua*) mislabelling, both across EU and non-EU member states, and in relation to a country's provision of cod products, be it primarily through internal landings or imports. Mislabelling is compared across countries, and the influence of legal, political and social factors that could either be permitting or preventing its proliferation is examined.

2. Methods

2.1. Selection of countries

To examine the incidence of cod mislabelling across Europe and to assess whether legislation and/or national cod production

influences seafood fraud, countries were selected based on their geographical location (bordering the North Atlantic and adjacent seas), on their EU affiliation (EU/non-EU) and national cod production (Table 1).

The Total Allowable Catch (TAC) of cod for each country was used as a proxy for production. TAC values for 2011 and 2012 were collated from European Commission publications [36,37] and the mean was obtained. Countries with an annual TAC > 15,000t were considered as ‘cod-producing’ (P⁺), while a TAC of < 4000t/annum, determined a low production (‘cod-importing’, P⁻) country (Table 1). Cod quotas for Atlantic Canada were sourced from Fisheries and Oceans Canada (<http://www.dfo-mpo.gc.ca>).

Packaged cod products were selected for sample collection due to their consistent availability throughout many countries, including those investigated. Packaged products also enable the identification of the supplier of a particular sample, when an EU approval barcode is present. The latter is required on all packaged fresh fish products sold in the EU to meet traceability requirements [38]. This approval number is a code that allows identification of the processing factory that handled the product prior to its delivery to the retailer. A list of these codes and associated processing companies, as well as their locations within the EU can be accessed online [39].

Large supermarket chains were selected in order to maximise sampling standardisation. Countries were chosen based on EU affiliation and national cod production for the following reasons: i) EU countries are subject to exhaustive, overarching regulations in terms of fishery trade and management, ii) the manner in which legislations are implemented and the quality of enforcement are the member states' responsibility and thus may vary between countries; iii) many cod stocks in EU waters are subject to quota partitioning among member and associate states, and some have been seriously depleted [40]. Overall, EU membership was employed as a predictor to assess whether belonging to a nation under transnational governance could influence the prevalence of mislabelling.

With regard to production, it may be hypothesised that the economic incentive to mislabel seafood in exporting countries is lower compared to countries that may not have such a thriving industry (importer), or it could be that importing seafood adds steps to the supply chain which may not be strictly regulated and may increase the opportunity for substitution as a result.

2.2. Sample collection

Between 43 and 53 cod products were obtained from different large supermarket chains in a major city in each of nine countries: the United Kingdom, UK; the Netherlands, NL; Belgium, BE; Denmark, DK; Norway, NO; Sweden, SE; Estonia, EE; Iceland, IS, Canada, CA. For Canada and the UK, Guelph and Reading, two smaller towns in the vicinity of Toronto and London, respectively, were sampled in addition to the latter in order to target a higher

Table 1

Description of countries sampled and number of stores visited. Abbreviation P⁻ denotes low cod production, P⁺ denotes high production.

Country and city	EU affiliation, Production	Number of supermarket chains sampled	Number of individual stores sampled	Total number of samples
United Kingdom, London	EU, P ⁺	7	19	43
Denmark, Copenhagen	EU, P ⁺	6	31	43
Sweden, Stockholm	EU, P ⁺	5	19	45
Norway, Bergen	Non-EU, P ⁺	6	20	43
Iceland, Reykjavik	Non-EU, P ⁺	6	6	53
Canada, Toronto	Non-EU, P ⁺	8	18	44
Estonia, Tallinn	EU, P ⁻	8	16	43
The Netherlands, Rotterdam	EU, P ⁻	7	26	44
Belgium, Brussels	EU, P ⁻	5	18	43

diversity of retailers. Sampling locations consisted of different stores of the main supermarket chains respective to each country (Table 1).

A variety of cod products were purchased, including breaded, frozen, fresh fillets, smoked, dried and salted, tinned and marinated products, reflecting country-specific consumer preferences and market availability, as well as integrating production chains of varying lengths, depending on the degree of processing and place of origin. Muscle tissue samples were taken and preserved in 100% ethanol and stored initially at room temperature and subsequently refrigerated.

Label information was recorded, including, where present, the EU approval number. The supplier origin of the samples was determined using these codes as in [16]. Retailer and supplier identity was subsequently coded with numbers (1, 2, 3...) and letters (A, B, C...), respectively.

2.3. DNA analysis

Total genomic DNA was extracted using a modified salt extraction protocol [41]. Tinned samples were subjected to an extra step, whereby the tissue sample was rinsed with double-distilled H₂O and blotted on paper to remove excess oil before DNA extraction. Approximately 630 base pairs from the 5' region of the COI gene were amplified through PCR. Total reaction volumes were 25 µl, containing 2 µl of the extracted DNA (25 ng/µl), 1 µl of each universal fish primers Fish F2 (10 µM) and Fish R2 (10 µM) [31] (Table 2), 2.5 µl of the 10x PCR Rxn buffer, 0.5 µl of dNTPs (deoxyribonucleotide triphosphates; 10 mM), 1.25 µl of MgCl₂ (50 mM), 0.2 µl of Platinum Taq polymerase, and 16.55 µl of MilliQ-H₂O. A negative control was included in all reactions. Amplifications were performed in a Biometra T300 Thermocycler according to [34]. PCR products were run by electrophoresis on a 1% agarose gel for visualisation and then purified through the addition of exonuclease I (exoI) and shrimp alkaline phosphatase (SAP). ExoSAP reactions consisted of 0.06 µl of exonuclease I (10U/microlitre), 0.6 µl of SAP (1U/microlitres) and 5.34 µl of MilliQ-H₂O. Cycling conditions were 15 min at 37 °C followed by 15 min at 80 °C and 30 min at 10 °C. Purified PCR products were sequenced uni-directionally by Macrogen Europe (Macrogen, Amsterdam, the Netherlands) using Sanger sequencing methods.

Canadian samples were processed at the University of Guelph, Biodiversity Institute of Ontario. DNA was extracted using the DNeasy Blood and Tissue kit (Qiagen, USA) following the manufacturer's guidelines. PCR reactions were performed in 12.7 µl volumes, which consisted of 10.5 µl mastermix, 0.1 µl of each FishF2_t1 and FishR2_t1 appended with M13 tails [42] (Table 2) and 2 µl of template DNA. PCR products were visualized on a 2% agarose gel using an E-Gel96 Pre-cast Agarose Electrophoresis System (Invitrogen) and uni-directionally sequenced using the BigDye Terminator version 3.1 Cycle Sequencing Kit (Applied Biosystems, Inc.) on an ABI 3730 capillary sequencer (see [43] for details). PCR amplification reactions were conducted on an Eppendorf Mastercycler ep gradient thermal cycler (Brinkmann Instruments, USA).

Sequencing reactions were conducted in 14.5 µl volumes, consisting of 1 µl M13F [42] (Table 2), 1 µl 5x Buffer, 1 µl BigDye, 10 µl ddH₂O and 1.5 µl PCR product. The sequencing reaction thermocycling conditions consisted of 2 min at 96 °C, followed by 30 cycles of 30 seconds at 96 °C, 15 seconds at 55 °C and 4 min at 60 °C, followed by a hold at 4 °C.

Obtained sequences were visualized in FinchTV, version 1.4 (Geospiza Inc., Seattle, WA, USA). Sequences were then entered into the Barcode of Life Data Systems online (BOLD species database, Biodiversity Institute of Ontario, University of Guelph, Guelph, Ontario, Canada; www.barcodinglife.org) to identify the species of each sample, then cross-referenced using BLAST on GenBank (Basic Local Alignment Search Tool, National Center for Biotechnology Information, Bethesda, Maryland; www.ncbi.nlm.nih.gov/). The 'species'-level identification function of the BOLD Identification Engine was used, and a sequence was assigned to a species when it matched database specimens with at least 99% similarity to avoid false positives [21]. Finally, the species identification generated was compared to the common or Latin name listed on the original label to determine whether the product was accurately labelled. Although Alaskan pollock, *Theragra chalcogramma*, has recently been placed in the genus *Gadus* to become *Gadus chalcogrammus*, it is sold under the market name "pollock", or "Alaska pollack" and it is not (yet) a species that can be sold as "cod". For products that were listed only as "cod", *Gadus macrocephalus* and *Gadus morhua* were permitted. Additionally, in cases where origin was listed, only one species was considered accurately labelled, e.g. when buying a fillet of cod from a fishmonger that listed "Canada East coast" as origin, only *Gadus morhua* was accepted, as *Gadus macrocephalus* is found in the Pacific. Five breaded products from Estonia were labelled as containing "tursalised", i.e. Gadiformes, therefore gadiform species other than *Gadus morhua* and *G. macrocephalus* were also accepted.

2.4. Statistical and phylogenetic analysis

Two-tailed Fisher's exact test was used in GraphPad Software (<http://graphpad.com>) to compare the level of mislabelling across countries.

Subsequently, a General Linear Model (GLM) was performed in SPSS version 20 (IBM, Chicago, IL) to test for the effects of three predictors on the level of mislabelling. Predictor variables were "EU status" (EU/non-EU), "Production" (high/low production), and "Product type" (fresh/processed), while the response variable was "Labelling" (mislabelled/correctly labelled). To test for the influence of EU affiliation, three EU (SE, DK, UK) and three non-EU (CA, NO, IS) countries were used, all of them high producers. To test for Production, three high production (DK, SE, UK) and three low production (EE, NL, BE) countries were used, all of them EU nations. In addition, "Country" was used as a nested predictor variable in both models.

To examine whether processed products were more prone to mislabelling, the factor "Product type" was included.

Table 2
PCR primer sets used to amplify COI. M13 tails are highlighted when present.

Name	Primer sequence 5'–3'	Reference
FishF2	TCGACTAATCATAAAGATATCGGCAC	[31]
FishR2	ACTTCAGGGTGACCGAAGAATCAGAA	[31]
FishF2_t1	TGTAAACGACGCCAG TCGACTAATCATAAAGATATCGGCAC	[42]
FishR2_t1	CAGGAAACAGCTATGAC ACTTCAGGGTGACCGAAGAATCAGAA	[42]
M13F	TGTAAACGACGCCAGT	[44]

Processed products included tinned, breaded, smoked and marinated products. For this analysis, all nine countries were included and country identity was also used as a nested predictor variable.

Furthermore, in countries where mislabelling was present, supplier and retailer identity was determined, coded with a number or a letter, and used in a chi-square (χ^2) contingency table to assess their effect on mislabelling and attempt to establish the level at which mislabelling could be taking place. Only EU barcodes were considered; other barcodes originating from outside Europe, such as those beginning with CN, i.e. China, were excluded.

A neighbour-joining (NJ) tree of Kimura two parameter (K2P) distances was created [45] to provide a graphic representation of the genetic divergence among the species detected through DNA barcoding. The robustness of topology nodes was tested by the bootstrap method with 1000 iterations. All sequence alignments were performed using ClustalW in Mega 5.2.2 software.

3. Results

3.1. Availability of and differences in cod products

There were differences in the type of products available in each country. Fresh fillets of cod were readily available in all countries, except in Estonia where they were rare, smoked and other types of processed cod being more common. In Norway, dried and salted cod (clipfish) was also common. Although Eastern Canada is home to Atlantic cod (*Gadus morhua*), its Pacific counterpart, *Gadus macrocephalus*, was more commonly encountered.

3.2. Level of mislabelling between countries

A total of 401 samples were purchased, 386 samples were PCR-amplified successfully and generated a $\geq 99\%$ match to a sequence in BOLD and BLAST. Three Estonian samples, all of which were labelled as “Pacific cod jerky”, returned a $\geq 99\%$ match to *Liparis*

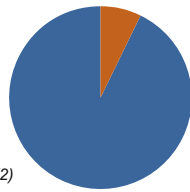
a) Non-EU countries / high production

Canada

N = 41

7.3%
92.7%

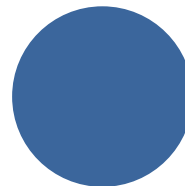
P. flavescens (1)
G. macrocephalus (2)



Norway

N = 43

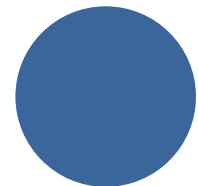
100%



Iceland

N = 53

100%

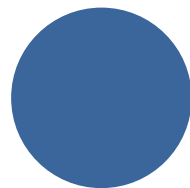


b) EU countries / low production

Netherlands

N = 44

100%

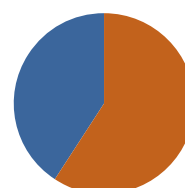


Estonia

N = 32

59.4%
40.6%

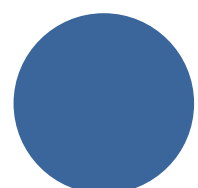
P. virens (8)
M. hubbsi (4)
Liparis spp. (3)
G. chalcogrammus (2)
A. minor (1)
M. aeglefinus (1)



Belgium

N = 43

100%



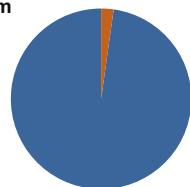
c) EU countries / high production

United Kingdom

N = 42

2.4%
97.6%

M. aeglefinus (1)

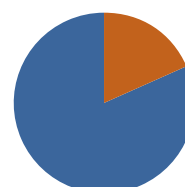


Denmark

N = 43

18.6%
81.4%

M. aeglefinus (8)

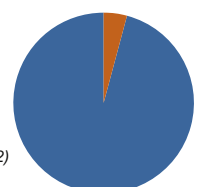


Sweden

N = 45

4.4%
95.6%

M. aeglefinus (2)



■ Mislabelled ■ Correctly labelled

Fig. 1. Proportion (%) and species identity of samples found to be mislabelled and correctly labelled. Numbers in brackets represent the number of mislabelled products. a) Non-EU countries, high production; b) EU countries, low production; c) EU countries, high production.

Table 3
All samples found to be mislabelled. BOLD, Barcode of Life Data Systems; BLAST, Basic Local Alignment Tool. Abbreviations represent the following genera: *Gadus*, *Pollachius* when preceding *virens*, *Melanogrammus* when preceding *aeglefinus*, *Merluccius* when preceding *hubbsi*, *Perca* when preceding *flavescens*, *Anarhichas* when preceding *minor*.

Sample	Location	Product description	Species	Origin	Bold Species ID	% match	BLAST ID	% match
UK11	London	Cod and chorizo fishcake	<i>G. morhua</i>	NE Atlantic (FAO 27), Norway and Iceland	<i>M. aeglefinus</i>	99.8	<i>M. aeglefinus</i>	99
DK11	Copenhagen	Cod fillet	<i>G. morhua</i>	FAO 27	<i>M. aeglefinus</i>	99.8	<i>M. aeglefinus</i>	99
DK14	Copenhagen	Cod fillet (fishmonger)	<i>G. morhua</i>	FAO 27	<i>M. aeglefinus</i>	100	<i>M. aeglefinus</i>	99
DK30	Copenhagen	Cod fillet	<i>G. morhua</i>	FAO 27	<i>M. aeglefinus</i>	99.8	<i>M. aeglefinus</i>	99
DK31	Copenhagen	Cod fillet	<i>G. morhua</i>	FAO 27	<i>M. aeglefinus</i>	100	<i>M. aeglefinus</i>	99
DK35	Copenhagen	Cod fillet	<i>G. morhua</i>	FAO 27	<i>M. aeglefinus</i>	100	<i>M. aeglefinus</i>	99
DK36	Copenhagen	Cod fillet	<i>G. morhua</i>	FAO 27	<i>M. aeglefinus</i>	100	<i>M. aeglefinus</i>	99
DK37	Copenhagen	Cod fillet	<i>G. morhua</i>	FAO 27	<i>M. aeglefinus</i>	100	<i>M. aeglefinus</i>	99
DK38	Copenhagen	Cod fillet	<i>G. morhua</i>	NE Atlantic	<i>M. aeglefinus</i>	100	<i>M. aeglefinus</i>	99
SE19	Stockholm	Cod fillet (fishmonger), skinless			<i>M. aeglefinus</i>	100	<i>M. aeglefinus</i>	99
SE27	Stockholm	MSC, frozen cod fillet, KRAV	<i>G. morhua</i>	NE Atlantic/Barents Sea (FAO 27)	<i>M. aeglefinus</i>	100	<i>M. aeglefinus</i>	99
EE6	Tallinn	Pacific cod jerky, salted, dried	<i>G. macrocephalus</i>	Pacific Ocean	<i>Liparis</i> spp.	100	<i>Liparis</i> spp.	100
EE10	Tallinn	Marinated cod (in water)			<i>M. hubbsi</i>	100	<i>M. hubbsi</i>	100
EE12	Tallinn	Marinated cod (in water)			<i>M. hubbsi</i>	99.8	<i>M. hubbsi</i>	99
EE14	Tallinn	Smoked cod	<i>G. morhua</i>	North Atlantic FAO 27 (Norway)	<i>P. virens</i>	100	<i>P. virens</i>	100
EE17	Tallinn	Frozen breaded cod fillet with spinach	<i>G. morhua</i>	NE Atlantic FAO 27, Norway	<i>G. chalcogrammus</i>	99.7	<i>G. chalcogrammus</i>	99
EE20	Tallinn	Smoked cod (fishmonger)	<i>G. morhua</i>		<i>P. virens</i>	100	<i>P. virens</i>	99
EE23	Tallinn	Cod fillets in oil, tinned		Baltic	<i>A. minor</i>	100	<i>A. minor</i>	99
EE24	Tallinn	Pacific cod jerky, salted, dried	<i>G. macrocephalus</i>	Pacific Ocean	<i>Liparis</i> spp.	100	<i>Liparis</i> spp.	99
EE25	Tallinn	Smoked cod	<i>G. morhua</i>	North Atlantic FAO 27, Norway	<i>P. virens</i>	100	<i>P. virens</i>	99
EE27	Tallinn	Smoked cod (fishmonger)			<i>P. virens</i>	99.8	<i>P. virens</i>	99
EE28	Tallinn	Marinated cod (in water)			<i>M. hubbsi</i>	100	<i>M. hubbsi</i>	100
EE30	Tallinn	Smoked cod	<i>G. morhua</i>	North Atlantic FAO 27, Norway	<i>P. virens</i>	100	<i>P. virens</i>	100
EE31	Tallinn	Smoked cod (fishmonger)	<i>G. morhua</i>		<i>P. virens</i>	100	<i>P. virens</i>	100
EE33	Tallinn	Smoked cod	<i>G. morhua</i>	North Atlantic FAO 27, Norway	<i>P. virens</i>	100	<i>P. virens</i>	99
EE35	Tallinn	Smoked cod	<i>G. morhua</i>	North Atlantic FAO 27, Norway	<i>P. virens</i>	100	<i>P. virens</i>	99
EE36	Tallinn	Cod fillet (fishmonger)			<i>M. aeglefinus</i>	100	<i>M. aeglefinus</i>	99
EE37	Tallinn	Marinated cod (in water)			<i>M. hubbsi</i>	100	<i>M. hubbsi</i>	100
EE39	Tallinn	Frozen breaded cod fillet with spinach	<i>G. morhua</i>	NE Atlantic FAO 27, Norway	<i>G. chalcogrammus</i>	99.8	<i>G. chalcogrammus</i>	99
EE43	Tallinn	Pacific cod jerky, salted, dried	<i>G. macrocephalus</i>	Pacific Ocean	<i>Liparis</i> spp.	99.8	<i>Liparis</i> spp.	99
CA1	Guelph	Large cod fillet, previously frozen		USA West Coast	<i>P. flavescens</i>	100	<i>P. flavescens</i>	
CA7	Guelph	Salted Atlantic cod			<i>G. macrocephalus</i>	100	<i>G. macrocephalus</i>	99
CA37	Toronto	Premium cod fillet		Canada East coast	<i>G. macrocephalus</i>	99.8	<i>G. macrocephalus</i>	99

agassizii, *Liparis chefuensis* and *Liparis tanakae*, thus could only be identified to genus level. Fifteen samples, the majority of which were tinned samples from Estonia, failed to amplify, due to the fragmented nature of DNA from highly processed products [46].

We found that 8 out of 43 (18.6%) fresh cod fillet samples from Denmark were mislabelled (Fig. 1). In addition, there were 2 out of 45 (4.4%) fresh samples from Sweden that were also incorrectly labelled, while one out of 42 cod products from the United Kingdom did not contain cod. The substitutive species in all the above samples was haddock, *Melanogrammus aeglefinus* (Fig. 1).

Three out of 41 (7.3%) samples from Canada were mislabelled, one of which contained yellow perch, *Perca flavescens*, the other two containing Pacific cod instead of Atlantic cod (Fig. 1). In contrast, there was no mislabelling in samples from Norway, Belgium, Iceland or the Netherlands (Fig. 1).

Estonian samples showed a high level of mislabelling with 19 out of 32 (59.4%) products containing a species other than cod (Fig. 1). The fraudulent products contained a diversity of substitute species, including snailfish species (genus *Liparis*), spotted wolffish (*Anarhichas minor*), saithe (*Pollachius virens*), Argentine hake (*Merluccius hubbsi*) and Alaska pollock (*Gadus chalcogrammus*). *Pollachius virens* was found exclusively in smoked products. A list containing the detailed description of all mislabelled samples and their sequence match is presented in Table 3. Furthermore, the phylogenetic relationships among the species detected are shown in Fig. 2.

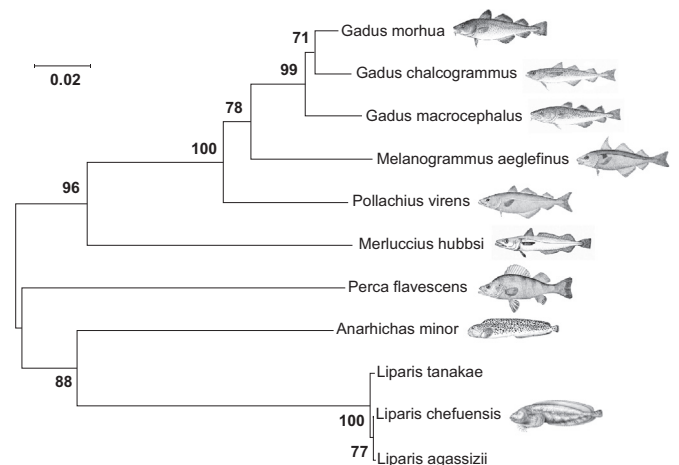


Fig. 2. Phylogenetic relationships among the species found across a range of "cod" products based on the COI barcoding gene. Bootstrap values > 70 are reported on the tree.

3.3. Factors potentially influencing mislabelling

3.3.1. EU affiliation, Cod production and Product type

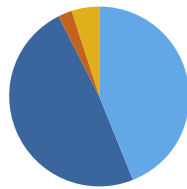
The level of mislabelling was compared across countries using Fisher's exact test. Estonia was significantly different from all other

a) Non-EU countries / high production

Canada

N = 41

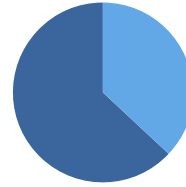
18
20
1
2



Norway

N = 43

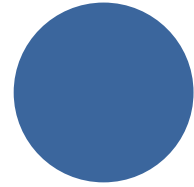
16
27



Iceland

N = 53

53

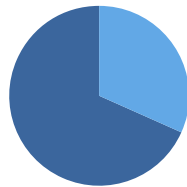


b) EU countries / low production

Netherlands

N = 44

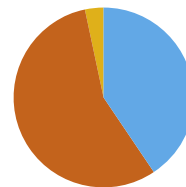
14
30



Estonia

N = 32

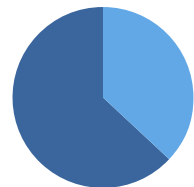
13
18
1



Belgium

N = 43

16
27

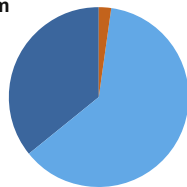


c) EU countries / high production

United Kingdom

N = 42

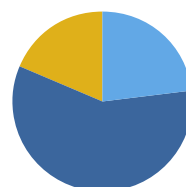
26
15
1



Denmark

N = 43

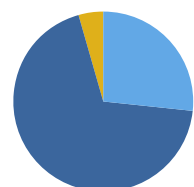
10
25
8



Sweden

N = 45

12
31
2



Correctly labelled / processed Correctly labelled / non-processed Mislabelled / processed Mislabelled / non-processed

Fig. 3. Number of mislabelled and correctly labelled processed and non-processed samples. a) Non-EU countries, high production; b) EU countries, low production; c) EU countries, high production.

eight countries, while Denmark was significantly different to all countries, except Canada.

Neither EU affiliation nor Production had a significant effect on mislabelling. The effect of “product type” was significant only for Estonian samples ($df=1$, $p=0.001$).

Although not significant, the factor “Country” was nevertheless found to have more of an effect than the factors of EU status, Production and Treatment. Fig. 3 gives a representation of the proportion of mislabelling in all processed and non-processed samples.

3.3.2. Retailer and supplier identity

Retailer and supplier origin were determined in countries where mislabelling was present, i.e. the UK, Denmark, Estonia, Sweden and Canada. For the latter, only retailer identity was examined as a barcode tracing back to the supplier or distributor was not present on labels. Samples from Denmark came from six suppliers, UK samples originated from twelve suppliers, those

from Sweden from eleven suppliers, and finally Estonian samples from ten suppliers (Fig. 4). In Denmark, all mislabelled samples which displayed an EU barcode originated from a single supplier (Supplier E; Fig. 4) and all but one from a single retailer. Fraudulent Estonian samples could be traced back to seven different suppliers and seven retailers (Fig. 4). In fact, only one retailer did not sell any mislabelled cod (Retailer 1, Fig. 4), while all products from Retailers 5 and 8 were mislabelled. However, it is important to bear in mind that in some instances the sample size per retailer or supplier was low and results should be interpreted with caution. With regard to Canada, mislabelled samples originated from three retailers. Both supplier and retailer identity had a significant effect on mislabelling in Denmark ($\chi^2=40.0$, $p<0.001$ and $\chi^2=37.3$, $p<0.001$, respectively), while in Estonia only supplier identity was significant ($\chi^2=22.0$, $p=0.005$). The effect of supplier/retailer identity was not significant for Sweden, the United Kingdom or Canada.

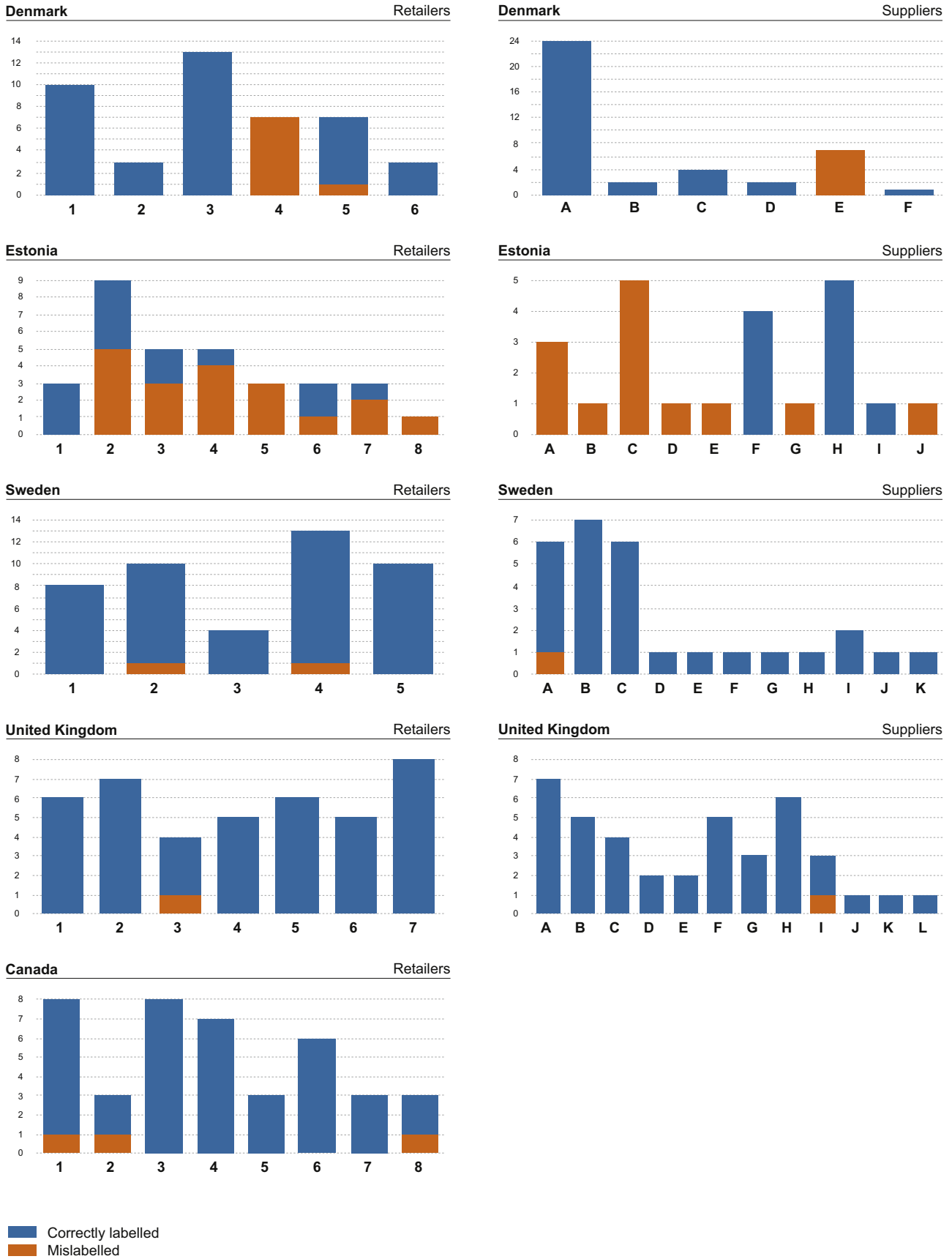


Fig. 4. Number of correctly labelled and mislabelled samples per retailer and supplier for Denmark, Estonia, Sweden, the United Kingdom and Canada.

4. Discussion

Species substitution is a widespread phenomenon, driven by resource scarcity and economic incentive. To date, most studies have tended to have a descriptive slant and focused on a region [12,19,28,47]. The present study investigates cod mislabelling on an unprecedented geographical scale, and goes beyond many previous investigations by establishing its prevalence across Europe and Canada, as well as attempting to assess whether EU affiliation and national cod production can influence its prevalence.

4.1. EU affiliation and cod production

The analysis showed that neither EU affiliation nor cod production had a significant effect on the level of mislabelling. Although not statistically significant, country identity had a greater effect on mislabelling than any of the other factors, and thus may play a greater role in seafood market dynamics. It is possible that, given the rapidly decreasing level of cod mislabelling [35], it would be necessary to further expand the geographical scope and sampling effort to increase the power to detect significant effects. Nevertheless, the current analysis represents the most comprehensive effort to date, on a species that arguably offers the greatest opportunity for transnational comparison of policy compliance.

Differences were present in several respects, the most striking being the varying level of mislabelling among countries. Estonia and Denmark, in particular, stand out with a staggering 59.4%, and 18.6% mislabelling, respectively (Fig. 1).

Because processing can mask the appearance of fish flesh and remove diagnostic characteristics, it can be expected that mislabelling would be more prevalent in products that have been processed to a higher degree. Findings from previous studies suggest that processed samples are more often mislabelled [16,20]. Our results show that this expectation is only supported by the data from Estonia, where many processed samples were fraudulent. On the other hand, the remaining mislabelled samples originating from Denmark and Sweden and the majority of those from Canada were fresh fillets, showing that the latter are also susceptible to mislabelling (Fig. 3).

Labelling specifications for fish and fish products in Canada are only required to include the common name of the species, while the country of origin is solely required on imported fish products. EU labelling legislation is comparatively stricter, indeed, it requires both the commercial and scientific name, the production method, catch area, country of origin and production, and, as of December 2014, the business name of the food operator and the fishing gear used [48]. However, even though progress in the development of traceability for seafood in Canada has been slower than in the EU, there are considerable movements underway to meet rising regulatory and market expectations.

4.1.1. Retailer and supplier behaviour

Out of a total of eight mislabelled samples from Denmark, all but one came from one particular retailer (Retailer 4, Fig. 4). In fact, 100% of samples purchased from Retailer 4 were mislabelled. This supports the idea that mislabelling can take place on a retailer level [16]; those seven samples also displayed an approval barcode that was traced back to a single supplier (Supplier E, Fig. 4), so investigating the matter further could provide clearer and more conclusive evidence as to which party is most responsible for mislabelling. It would be beneficial to investigate whether Supplier E provides other retailers with its products and to establish whether those are also incorrectly labelled. Inversely, if Retailer 4 sources its cod from other suppliers, it could be interesting to analyse those samples. In the case of Estonia, supplier-level mislabelling appears likely, as retailers sell both mislabelled and

correctly labelled cod, while mislabelled samples are exclusively provided by a number of suppliers (Suppliers A, B, C, D, E, G and J; Fig. 4). However, this should only be taken as an indication of responsibility; results should be interpreted with caution as the sample size per supplier is low and does not allow for unambiguous conclusions.

4.1.2. Substitute species

Substitute species included commonly substituted gadiform species, such as *Melanogrammus aeglefinus* (haddock), *Merluccius hubbsi* (Argentine hake), *Pollachius virens* (saithe) but also included species well outside of the Gadiformes (Fig. 2), such as *Liparis* spp (Order Scorpaeniformes), which are not highly commercial species. This seemingly systematic substitution for cheaper species and sometimes species that are not part of a targeted fishery, suggests that mislabelling is deliberate, using species that are available, irrespective of identity or similarity to gadoids. In contrast, all smoked products contained *P. virens*; it thus appears that the choice of substitute species may be driven by the type of product to some extent. Several studies have provided evidence of species substitution for economic gain [15,21]. Mislabelled Danish, Swedish and British samples in this study were substituted with haddock, *Melanogrammus aeglefinus*. The reason for this substitution is not evident. Haddock is generally only marginally less expensive than cod, in fact, relatively recently, haddock prices have been on the increase since ICES recommended a drop in TAC, making 2014 catches less than half the 2012 levels [49]. Reasons for this substitution may not be the general lesser-value species sold as a higher value species; rather it could be that fishermen were over the quota for haddock and therefore attempted to disguise it as cod; or alternatively, it may simply reflect processing errors at the factory level.

4.1.3. Cultural, legal and economic constraints

Norway and Iceland show no mislabelling and it is worth examining their fisheries management systems. Both countries are members of the European Economic Area (EEA) rather than the European Union, and consequently their fisheries are subject to single rather than multi-jurisdictional management [50]. Additionally, a study has revealed that Icelandic and Norwegian fisheries have been subject to lower levels of political adjustment than those managed by the European Commission [51]. Areas more prone to adjustment included the Baltic Sea, which, incidentally, is Estonia's main fishing grounds. Perhaps most importantly, Norway and Iceland exploit the Northeast Arctic and Icelandic cod stocks, which support the largest cod fisheries [52]. Cod landings for Norway and Iceland exceed that of all other countries [53], and both countries have a small population and act as the major suppliers of fishery products to the EU [54], thereby removing incentives for domestic cod mislabelling.

While Iceland is practically in sole control of its cod fishery, and Norway shares its cod stock with Russia and decides on a harvesting policy jointly with Russia [55], The Netherlands, Sweden, the United Kingdom, Belgium and Denmark are among the EU countries exploiting the North Sea cod fishery, under the EU quota system; yet, only Denmark exhibits notable levels of mislabelling, indicating that country-specific governance dynamics play a major role in determining market transparency.

This study revealed interesting differences in country-specific prevalence and type of cod mislabelling across several countries and provides scope for further examination. The high level of mislabelling in two of the countries provides further evidence that the phenomenon is still present and even though it has been over a decade since the first mislabelling study [56], it appears that government tools to combat fraud are not quite flawless, and it is still possible to exploit loopholes in the current legislation.

On a positive note, in Europe, the recent adoption of more exhaustive labelling information [8] is likely to be of added benefit to allow consumers to make informed, sustainable seafood choices. Beyond Europe, in the US, the FDA has recently introduced a programme called Fish Seafood Compliance and Labelling Enforcement (Fish SCALE, <http://www.accessdata.fda.gov/FDATrack/track-proj?programme=cfsan&id=CFSAN-ORS-FishScale>). This involves the development and implementation of regulatory genetic methods that will allow the FDA, other regulatory agencies, and the seafood industry to confirm seafood labelling and identify at which step in the supply chain violations may be occurring. The FDA's Seafood List currently holds DNA sequences for the most important commercial species. Furthermore, the recently established Presidential Task Force to fight IUU (http://www.nmfs.noaa.gov/ia/iuu/noaa_taskforce_report_final.pdf) also suggests forthcoming positive change in the United States.

5. Conclusions

Our findings suggest that country-specific dynamics may play a bigger role in driving species substitution than any other factor; however, this applies to a broadly traded and exhaustively investigated commodity and cannot be extrapolated to the myriad of other seafood products on the market. It would be valuable to assess the extent to which other commercial species are also subject to fraudulent practices, and how these compare to the situation with cod, whose product labels have recently appeared to be rather accurate [57]. It would also be interesting to examine further the supply chains in those countries with higher mislabelling, in order to establish potential points of error and/or specific loopholes in national enforcement. Alternatively, since more than half of the countries examined showed no or very low levels of mislabelling, examining the characteristics of those nations' fishery sectors could provide leads as to where and how to implement control measures in the seafood supply chain.

Seafood fraud not only hinders consumers' ability to make informed and sustainable seafood purchases, it also harms fisheries and fishermen by facilitating the laundering of illegally caught seafood products into the market. Several strategies exist to mitigate the incidence of mislabelling and improve food quality control and traceability at all stages in the chain of production. These include the introduction of incentives to comply with regulations, improved monitoring, and the required use of genetic identification techniques, such as DNA barcoding, as a regulatory tool. It is nevertheless crucial to bear in mind that management and legislative tools can only be as good as their monitoring and enforcement systems.

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