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Current Methods for Seafood Authenticity Testing in Europe: is there a Need for Harmonisation?

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Abstract:

Mislabelling of food products has recently received a great deal of public scrutiny, but it remains unclear exactly what methods are being utilised in laboratories testing the authenticity of foods. In order to gain insight into the specific area of the analysis of seafood, a questionnaire focusing on the taxonomic groups typically analysed and the techniques utilised was sent to over one hundred accredited laboratories across the UK, Ireland, Spain, Portugal, France and Germany. Forty-five responded positively, demonstrating significant differences in both the species analysed and methods utilised among the countries included in the survey. Indeed, a diversity of methods was employed across laboratories and efforts to harmonise and/or standardise testing were evident only at national scale. This contrasts with the EU wide scale of regulation on seafood labelling, and may lead to inconsistencies in the results produced in countries.

Highlights

- ▶ The first international survey of accredited food authenticity laboratories.
- ▶ Significant differences in the methods used in authenticating seafood.
- ▶ Significant differences in the main products monitored.
- ▶ Lack of harmonisation and standardisation across the EU (despite common regulation).
- ▶ Widespread uptake of DNA-based methods, particularly sequencing.

Keywords : Food testing ; Forensically informative nucleotide sequencing ; Species identification ; DNA barcoding ; Fisheries

58 1. Introduction

59 Mislabelling of food products, so that the description or labelling does not accurately reflect the
60 purveyed food, has recently received a large amount of public attention. This came to great
61 prominence during the 2013 “horse meat scandal” in Europe, where a range of supposedly beef
62 products were found to contain horse flesh (FSAI, 2013). What makes this discovery surprising is that
63 it took place despite the clear set of European Union (EU) regulations relating to food traceability
64 and labelling, which require a complex system of documentation and audit to ensure that food
65 remains authentic and traceable (Schröder, 2008). In fact, it was only through the use of DNA based
66 methodologies for identifying species that this food fraud was detected.

67

68 This case clearly demonstrates the utility of DNA based authenticity techniques as a tool in food
69 control, which have been shown to be particularly useful in the specific case of testing seafood,
70 particularly fish, due to astounding biological diversity that underpins this complex market. The
71 global trade of seafood products over the last 40 years increased from 0.8 million metric tons worth
72 \$1.3 billion in 1975 to 2.4 million metric tons worth \$16.5 billion in 2012 (NOAA, 2013). This has also
73 been accompanied by greater complexity in commodity flows, with some products crossing multiple
74 national boundaries during the supply chain, including movements into territories without stringent
75 traceability requirements (D`Amico et al, 2014). There is also a huge diversity of species and
76 products available on the global seafood market, such that the U.S. Food and Drug administration
77 includes approximately 1700 species of commercial finfish and shellfish in its Seafood list (FDA,
78 2013).

79

80 Whilst species identification can usually be made from morphological characteristics from fish in
81 their whole form, seafood is often processed before reaching the consumer. This potentially creates

82 a situation where substitution of species, particularly for economic gain (i.e. where a low value
83 product is substituted for a higher value one) may occur, but is difficult/impossible to identify
84 without authenticity testing. Indeed, numerous genetic studies have now been published that
85 demonstrate high levels of substitution and mislabelling across a variety of seafood products (e.g.
86 Griffiths *et al.* 2013; Rehbein & Oliveira, 2012; Miller & Mariani, 2010; Wong & Hanner, 2008).

87

88 These studies have clearly demonstrated that seafood mislabelling is a widespread phenomenon,
89 but they also highlight the huge diversity of methodologies that have been developed for
90 identifying/distinguishing between species. The exhaustive recent reviews by Rasmussen &
91 Morrissey (2008), Teletchea (2009) and Lago *et al.* (2013) emphasise the fact that many traditional
92 and official methods used in species identification are based on the biochemical analysis of specific
93 proteins, e.g. isoelectric focusing (IEF), high performance chromatography or immunoassay. These
94 approaches have a number of disadvantages, the most significant being that many can only be
95 applied to fresh samples, and they cannot be used on highly processed (i.e. cooked or canned
96 products) because the proteins become denatured upon heating. In comparison, DNA is a more
97 thermostable molecule and although it may become degraded during processing, short fragments
98 are generally recoverable and can form the basis of authenticity tests in processed foods (Quinteiro
99 *et al.*, 1998; Mackie *et al.*, 1999). Furthermore, detailed surveys of the scientific literature on
100 seafood authenticity reveal it to be a continuously evolving field, with over 150 peer-reviewed
101 papers on the topic in the period 1995-2008 (Rasmussen & Morrissey, 2009; Teletchea, 2009).

102

103 Against this backdrop of increasing regulatory complexity and technological development, it is
104 unclear what methods are actually being utilised in public and private authenticity laboratories. The
105 EU regulations concerning food labelling and traceability contain little/no guidance on what

106 techniques should be applied and no recognition of an “official” method. While the plethora of DNA
107 techniques available offers undeniable advantages in biological identification, their ability to
108 distinguish particular groups of species may vary considerably. Therefore, the aim of this study was
109 to survey public and private laboratories that conduct seafood authenticity testing across Europe to
110 investigate how products, particularly finfish products, are analysed. This knowledge is vital in a
111 regulatory and legal context as it remains key to accurately testing food and providing robust
112 evidence for prosecuting those that break the law. It also represents a fundamental step towards the
113 establishment of an efficient, validated, standardised transnational procedure for monitoring
114 authenticity in the seafood market.

115

116 **2. Materials & Methods**

117 *2.1 Survey Development*

118 In order to maximise the number of responses, a relatively simple questionnaire was constructed,
119 which included eight brief questions and avoided asking for commercially sensitive information that
120 could potentially have prevented response from commercial or private laboratories (supplementary
121 material 1). The key questions posed were;

122

123 •Are you a public or private testing facility?

124 •What species are you most commonly asked to check for mislabelling?

125 •What biochemical or molecular method(s) do you use for distinguishing between species in the
126 analysis of sea-foods?

127 •In any molecular genetic methods, what region of the DNA do you use (nuclear vs mitochondrial,
128 specific protein coding genes or non-coding regions; can you tell us which you use for each
129 taxonomic group)?

130 •Are you developing any novel approaches that you could tell us about in broad terms?

131

132 The remaining questions were related to the methodologies employed and generally concerned the
133 capabilities of the laboratories i.e. in terms of equipment, specificity of methods and the extent of
134 reference data collected.

135

136 The questionnaire was translated and e-mailed to 101 authenticity laboratories involved in the
137 testing of seafood in the United Kingdom (UK), Republic of Ireland (ROI), France, Germany, Spain and
138 Portugal, which include five of the top ten countries in Europe in terms of total supply of fisheries
139 products (FAO, 2009). The questionnaires were initially sent out in November 2012, with efforts to
140 elicit responses continuing until March 2013. How laboratories were short-listed in each country
141 varied, but efforts were made to contact both public and private authenticity facilities that have
142 some degree of officially recognised accreditation (e.g. from the United Kingdom Accreditation
143 Service, Portuguese Institute for Accreditation, National Association of German Chemists, Spanish
144 National Entity for Accreditation or French Committee of Accreditation). However, given the survey's
145 authors' experience in the field, questionnaires were also distributed through previously established
146 networks of contacts.

147

148 *2.2 Statistical Analysis*

149 Given that the amount of detail provided by different laboratories to many questions varied
150 considerably, efforts were made to standardise answers by grouping specific responses into broader
151 categories, prior to statistical analyses. Additionally, as single responses were gathered from
152 Portugal and ROI, these were combined with those from their geographically and culturally most
153 proximate neighbours; Spain and UK, respectively, for statistical testing.

154

155 To explore general patterns in the data, principal component analysis (PCA) was conducted in
156 PRIMER-6 (Clarke & Warwick, 2001), with each testing laboratory representing an individual data
157 point in the ordination. The software was also used to conduct a non-parametric analysis of
158 similarity (ANOSIM), utilising the Bray-Curtis distance measure. Specifically, the ANOSIM was used to
159 test if there were significant differences between countries in terms of the types of products/species
160 laboratories commonly test, and also for differences in the authenticity methods employed. The
161 hypothesis being that different countries will have cultural differences in the seafood products they
162 consume, leading to significant differences in the types of products analysed and the authenticity
163 methodologies tailored to them.

164

165 **3. Results**

166 Of the 101 laboratories contacted, 45 responded positively; a response rate of 44.6%. Across all the
167 countries included, a total of 30 completed questionnaires were gathered from public laboratories
168 and 15 from private facilities (summarised in supplementary material 2). The results for the
169 responses to the key questions are examined below, one at a time.

170

171 *What species are you most commonly asked to check for mislabelling?*

172 A total of 38 laboratories provided information on the species they typically test and the level of
173 detail provided varied considerably, some facilities listed Latin names, whilst others included much
174 broader commercial designations. Therefore, the responses were classified into wider taxonomic
175 groups, which also included species that are commonly used as substitutes e.g. the gadoid
176 classification includes any responses of: “cod”, “haddock”, “gadoids”, “white fish” and “pangasius”
177 or “panga”, as species from the tropical catfish Pangasiidae have widely been used as a substitute
178 for gadoids. A total of 18 classes were constructed (of which nine included only one or two records,
179 see supplementary material 3). By far the most commonly tested species groups were: gadoids, flat
180 fish, tunas and salmonids. Global comparisons were highly significant ($R = 0.447$, $p\text{-value} = 0.001$,
181 table 1), suggesting big differences between the countries in terms of the products and species
182 commonly tested. A simplified version of the dataset, with the species classes that only
183 incorporating one or two records removed, was analysed via PCA in order to reduce the number of
184 variable vectors and make the figure clearer (fig. 1, the PCA with all vectors is also included in
185 supplementary materials 4). It clearly demonstrates how testing in the UK, ROI and France is
186 dominated by gadoids and salmonids, whilst flat fish are more predominant in Germany and a
187 combination of hakes, clupeids and tunas are important in Spain and Portugal.

188

189 *What biochemical or molecular method(s) do you use for distinguishing between species in the*
190 *analysis of seafood?*

191 All 45 laboratories answered this question. Similar to above, the responses were grouped into 12
192 broader methodological classes (supplementary material 5). So for example, any sequencing based
193 identification methods, regardless of the gene/region targeted were classed under forensically
194 informative nucleotide sequencing (FINS). The three most widely utilised methods were FINS (in 31
195 laboratories), restriction fragment length polymorphism (RFLP, in 18) and IEF (in nine). This reflects
196 the fact the DNA-based methods were far more prevalent, with only 10 of the laboratories including

197 a biochemical protein-based protocol in their response. Global testing of the results demonstrated
198 significant differences between countries (ANOSIM; $R = 0.259$ $p = 0.001$, table 1). Further comparison
199 of the pairwise tests between countries shows that much of this result is due to the UK & ROI group
200 (for which all three tests against other groups were significant at the 95% confidence interval, table
201 1). A simplified version of the dataset, where classes of method with a single record were removed
202 in order to reduce the number of variable vectors, was analysed via PCA (fig. 2, the PCA with all
203 vectors is also included in supplementary materials 6). It shows the importance of FINS, RFLP and IEF
204 across Europe, but there is little evidence of different patterns in methodological application
205 between countries surveyed.

206

207 The survey included three further questions that are related to methods of choice. First, “are your
208 methods universal or tailored to specific groups of fish?” Thirty nine laboratories responded, two
209 said their methods were specific to certain groups, 17 utilised universal methods and 20 used both.
210 Second, “have you developed your own databases of reference material or baseline information to
211 distinguish between species?” All 45 laboratories responded, 11 exclusively employed public
212 databases, 16 utilised their own private reference data and 18 used both. Finally, “what key pieces
213 of equipment do you use in distinguishing between species?” The majority of laboratories that
214 responded to this question indicated they had access to basic molecular biology equipment e.g. PCR
215 machines, electrophoresis kit etc.

216

217 *In any molecular genetic methods, what region of the DNA do you use (nuclear vs mitochondrial,*
218 *specific protein coding genes or non-coding regions; can you tell us which you use for each taxonomic*
219 *group)?*

220 40 laboratories gave some indication of the DNA they target across the methodologies they utilise.
221 Only 10 of these specified that at least some of their methods utilised nuclear DNA, and these
222 involved a wide range of targets, both anonymous DNA regions e.g. microsatellites and randomly
223 amplified polymorphic DNA (RAPD) and specific genes e.g. rhodopsin and pantophysin
224 (supplementary material 7). Conversely, all 40 laboratories positively indicated mitochondrial DNA
225 was a focus of their authenticity testing. Three gene regions proved to be the markers of choice;
226 Cytochrome B (cyt-b, specified in 29 responses), cytochrome oxidase 1 (COI, in 11) and 16s
227 ribosomal DNA (16s, in 7). Global testing of the results failed to detect significant differences
228 between countries, although the result was very close to the 95% confidence interval (ANOSIM; $R =$
229 0.121 $p = 0.050$, table 1).

230

231 *Are you developing any novel approaches that you could tell us about in broad terms?*

232 Only two laboratories declined to answer this question, but the remaining 43 responses were
233 generally in the negative. Interestingly, of the nine laboratories who indicated the technologies they
234 were currently investigating, six specified “real time” PCR (rtPCR) based methods.

235

236 **4. Discussion**

237 This work represents the first effort to assess what methodologies are being applied to seafood
238 authenticity in Europe. There are some very clear patterns that emerge from the responses: firstly,
239 the groups of species tested across the regions varied significantly. Second, DNA based methods,
240 particularly FINS, dominated the responses, but approaches were inconsistent between laboratories,
241 and protein based biochemical methods are still commonly utilised. Although a total sample size of
242 45 laboratories does not necessarily provide enough data to investigate subtler dynamics within and
243 between countries, some patterns are very strong and reveal a substantial lack of standardisation.

244

245 This survey identified highly significant differences in the species commonly tested in the countries
246 surveyed, which is entirely consistent with the cultural preferences for seafood across these regions.
247 A range of white fish species are commonly consumed in northern France, UK and ROI, which is
248 clearly reflected in figure 1. Similarly, a culture of consuming hake and clupeids in Spain and Portugal
249 and the high value attached to sole (*Solea solea*) in Germany, are also reflected in the results. It
250 seems very likely that if further regions of Europe were surveyed—with their own traditions of
251 consuming seafood – even more complex patterns in the species tested would emerge (Armani *et*
252 *al.*, 2012a). This presents a significant issue in terms of harmonising and standardising approaches to
253 seafood authenticity across Europe, to which the EU is generally committed, and for which all
254 member states are governed by the same regulations regarding traceability and authenticity. It is
255 due to the fact that laboratories in various countries are likely to be more familiar with testing for a
256 discrete sub-set of species (and may have developed methods optimised to these groups), but any
257 standard methodology will have to function across a much broader taxonomic range that reflects
258 the diversity of cultural preferences in seafood consumption across the EU, and the progressively
259 more globalised import landscape of the EU (Sotelo & Pérez-Martín, 2007; De Silva, 2010; Armani *et*
260 *al.*, 2012b). Nevertheless, it is important to note that there are existing efforts to harmonise testing
261 of seafood, for example, the Food Analysis Performance Assessment Scheme (FEPAS;
262 <http://fapas.com>) provides a regular fish authenticity proficiency testing scheme. This involves the
263 analysis of “blind” samples, i.e. where the species of origin is unknown, which can be incorporated
264 into the requirements of national accreditation bodies. In Germany, harmonisation has gone a step
265 further; under the German Food and Feed Act (§64 Lebensmittel- und Futtermittelgesetzbuch), ring
266 trials have been used to develop a range of officially recognised standard methods, which are
267 coordinated by the Federal Office of Consumer Protection and Food Safety (BVL;
268 <http://www.bvl.bund.de>). However, this also emphasises how harmonisation has largely been driven
269 at a national, but not EU-wide, scale.

270

271 Despite the view that traditional and official methods used in species identification are based on the
272 analysis of specific proteins (Rasmussen & Morrissey, 2008; Teletchea, 2009), the majority of
273 authenticity laboratories routinely employ DNA based protocols in their analysis of seafood and have
274 access to equipment for basic molecular genetics. This can be seen as further evidence that
275 control laboratories, which are traditionally seen as being staffed by analytical chemists, are
276 embracing genetic tools for distinguishing species (Wolfe *et al.*, 2013). Nevertheless, it is important
277 to note that IEF is still commonly utilised, remaining the third most commonly employed class of
278 method. This probably relates to the low-cost and speed of the protocol (making it an ideal approach
279 for an initial screening of samples), and the long period it has been the Association of Analytical
280 Communities (AOAC) recognised method for species identification (AOAC, 1980).

281

282 The widespread use of DNA based methods does not necessarily mean that the protocols across the
283 laboratories are standardised. The surveyed laboratories utilised a diverse set of techniques and a
284 global test across all regions was significant, suggesting differences in the methods applied between
285 countries. In particular, the UK and ROI group was generally identified as using a distinct set of
286 methods to those in the rest of Europe. This reflects previous efforts of the Food Standards Agency's
287 Food Authenticity Programme to transfer DNA methodologies to UK Official Food Control
288 laboratories. They supported knowledge transfer activities and provided funding for a standard
289 operating procedure (SOP) based on a RFLP protocol on a lab-on-a-chip platform (Agilent 2100
290 Bioanalyser) for species identification (Dooley *et al.*, 2010; Garrett *et al.*, 2010). So the majority of
291 public analyst laboratories follow the same SOP, probably making this one of the largest efforts to
292 standardise seafood authenticity testing with a single method in Europe. However, the use of a RFLP
293 technique in the UK contrasts with the more widespread application of FINS in the rest of Europe.

294 The PCA scatter also illustrates how the variation in techniques used is considerable, even within
295 most countries.

296 The grouping of protocols into broader methodological classes to facilitate the statistical analysis
297 actually disguises the full diversity of approaches revealed by the questionnaires. So for example,
298 FINS was the most commonly utilised class of method, but it includes many laboratories with
299 different target sequences, and even where laboratories utilise the same genetic region it does not
300 necessarily imply the same primers/protocol are being applied (Burgener & Hübner, 1998; Sevilla *et*
301 *al.*, 2007). The subsequent question in the survey, regarding the regions of DNA targeted, helps
302 clarify this issue to some degree. It demonstrates a relatively wide diversity of DNA targets, both
303 nuclear and mitochondrial, with mitochondrial regions dominating. This reflects several advantages
304 mitochondrial DNA presents in authenticity testing, particularly its haploid matrilineal inheritance
305 and its high copy number within the cell (Rasmussen & Morrissey, 2008; Teletchea, 2009). It is also
306 interesting to note that despite the global Barcode of Life initiative promoting the sequencing of COI
307 for identifying species (Ratnasingham & Hebert, 2007) and the US Food and Drug Administration
308 developing this as a validated method utilised for seafood authenticity, *cyt-b* remains the most
309 popular DNA target (although, since these responses refer to any DNA based method, not just FINS,
310 there may be a systematic inflation of the role of *cyt-b*, as this region has been long optimised for
311 RFLP analysis).

312

313 This survey revealed the diversity of biochemical and genetic methods that are used in laboratories
314 across Europe, demonstrating a general lack of standardisation in testing between laboratories. This
315 has important implications, essentially meaning that if the same sample was analysed in different
316 laboratories, conflicting results could be generated or, more likely, the majority of specific tests for a
317 narrow range of species/products will simply fail to identify the sample. However, inconsistency may
318 also arise when considering the reference data that is being utilised to perform species

319 identification. The survey specifically included a question concerning whether the laboratories
320 employed their own private, or a publically available, database (with some using only private or
321 public databases, and others a combination). Both kinds of databases could potentially be associated
322 with inconsistent identifications. Public databases tend to be the most comprehensive, but may
323 contain sequences erroneously attributed to the wrong species, which may require some
324 interpretation. Conversely, private data collections may differ significantly between laboratories,
325 producing an additional source of inconsistencies when comparing results. The use of reference
326 tissues is an aspect that will play a part in future improvements of method standardization. While
327 sequencing-based approaches rely on large amount of reference data stored in public data bases
328 (e.g. GenBank, <http://www.ncbi.nlm.nih.gov/>; BOLD, www.boldsystems.org) most other techniques
329 hinge on the existence of voucher specimens in the control labs, whose provision and exchange
330 would represent a challenge for a robust, wide-spectrum, long-lasting standardisation initiative.
331 Even the choice of sequence data bases in support of FINS should be based on the level of
332 maintenance, verification and filtering of the said sequences, in order to minimize the risk of “false
333 matches”, especially for less commonly traded species. Failure to consider these constraints will
334 have serious implications within the context of enforcement and prosecution, acting to undermine
335 the confidence of stakeholders.

336

337 **5. Conclusions**

338 Despite the view that most traditional methods used in species identification are based on the
339 biochemical analysis of specific proteins, DNA sequencing appears to be the most commonly applied
340 approach (with the analysis of the cytochrome-b gene dominating). However, there is a diversity of
341 approaches that highlights the lack of consistency in how protocols for identifying species in seafood
342 are applied at a European level. This absence of harmonisation and standardisation could lead to
343 inconsistencies in results generated between laboratories, which may have significant regulatory or

344 legal implications. These results underline the need for a more rigorous standard operating
345 procedure to be applied across the EU. Previous attempts to develop standard approaches to
346 seafood identification at a national scale have been successful, both within the UK (Wolfe et al.,
347 2013) and the US (Handy *et al.*, 2011), demonstrating the feasibility of such an approach.

348

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354

355 **References**

356 AOAC. (1980). Official Method 980.16 Identification of Fish Species: Thin-Layer Polyacrylamide Gel
357 Isoelectric Focusing Method. *Journal of AOAC International*, 63:69.

358

359 Armani, A., Castigliego, L., Tinacci, L., Gandini, G., Gianfaldoni, D., & Guidi, A. (2012a). A rapid PCR–
360 RFLP method for the identification of *Lophius* species. *European Food Research and Technology*, 235,
361 253-263.

362

363 Armani, A., Castigliego, L., & Guidi, A. (2012b). Fish fraud: The DNA challenge. *CAB Reviews* 2012 7,
364 No 071.

365

366 Burgener, M., & Hübner, P. (1998). Mitochondrial DNA enrichment for species identification and
367 evolutionary analysis. *Zeitschrift für Lebensmitteluntersuchung und-Forschung A*, 207, 261-263.

368

369 Clarke K. R., & Warwick R. M. (2001). Change in marine communities: an approach to statistical
370 analysis and interpretation, 2nd edn. Plymouth Marine Laboratory, Plymouth, UK.

371

372 D'Amico P., Armani A., Castigliero L., Sheng G., Gianfaldoni D., & Guidi. A. (2014). Seafood
373 traceability issues in Chinese food business activities in the light of the European provisions. *Food*
374 *Contol*, 35, 7-13.

375

376 De Silva, D. A. M. (2010). Value chain of fish and fishery products: origin, functions and application in
377 developed and developing country markets, Value chain project, Food and Agriculture Organisation
378 (FAO), Rome Italy. pp 7-10.

379

380 Dooley, J., Garrett, S., Sage, H., Clark, M., & Brown, H. (2010). Application of a chip-based capillary
381 electrophoresis system to enable simple PCR detection of fish species. Project Code: Q01069. Food
382 Standards Agency, Foodbase, Open Access Research Repository Available at;
383 http://www.foodbase.org.uk/results.php?f_report_id%95 (accessed 03.11.12)

384

385 FAO (Food and Agriculture Organisation). (2009). Yearbook of Fisheries Statistics Summary tables.

386 <http://www.fao.org/fishery/statistics/global-consumption/en> (accessed 06/02/2014)

387

388 FDA (Food and Drug Administration). (2013). The Seafood List FDA's guide to acceptable market
389 names for seafood sold in interstate commerce,
390 [http://www.fda.gov/food/guidanceregulation/guidancedocumentsregulatoryinformation/seafood/u](http://www.fda.gov/food/guidanceregulation/guidancedocumentsregulatoryinformation/seafood/ucm113260.htm)
391 [cm113260.htm](http://www.fda.gov/food/guidanceregulation/guidancedocumentsregulatoryinformation/seafood/ucm113260.htm) (accessed December 16, 2013).

392

393 FSAI (Food Safety Authority of Ireland). (2013). Horse meat used to cheat consumers. FSAI News
394 January/February 2013, Vol. 15, Issue 1. FSAI, Dublin.

395

396 Garrett, S., Dooley, J., Brown, H., & Clarke, M. (2010). Extending the fish species lab-on-a-chip
397 capillary electrophoresis PCR-RFLP database. Food Standards Agency, Foodbase, Open Access
398 Research Repository Project Code: Q01099, Available at;
399 http://www.foodbase.org.uk/results.php?f_report_id%532. (Accessed 03.11.12)

400

401 Griffiths, A. M., Miller, D. D., Egan, A., Fox, J., Greenfield, A., & Mariani, S. (2013). DNA barcoding
402 unveils skate (Chondrichthyes: Rajidae) species diversity in 'ray' products sold across Ireland and the
403 UK. *PeerJ*, 1, e129.

404

405 Handy, S. M., Deeds, J. R., Ivanova, N. V., Hebert, P. D., Hanner, R., Ormos, A., Weigt, L. A., Moore,
406 M. M., Hellberg, R. S., & Yancy, H. F. (2011). Single laboratory validated method for DNA-barcoding
407 for the species identification of fish for FDA regulatory compliance. *Journal of AOAC International*,
408 94, 201-210.

409

410 Hellberg, R. S. R., & Morrissey, M. T. (2011). Advances in DNA-based techniques for the detection of
411 seafood species substitution on the commercial market. *Journal of the Association for Laboratory*
412 *Automation*, 16, 308-321.

413

414 Lago, F. C., Alonso, M., Vietes, J. M., & Espineira, M. (2013). Fish and Seafood Authenticity – Species
415 Identification, In I. S., Bozaris (ed.), *Seafood Processing Technology, Quality and Safety*, (pp. 419-
416 440). John Wiley & Sons, Ltd, Chichester, UK.

417

418 Mackie I.M., Pryde S., González-Sotelo C., Medina I., Pérez-Martín R.I., Quinteiro J., Rey-Mendez M.,
419 Rehbein H. (1999). Challenges in the identification of species of canned fish. *Trends in Food Science*
420 *and Technology*, 10: 9-14.

421

422 Miller, D. D., & Mariani, S. (2010). Smoke, mirrors, and mislabeled cod: poor transparency in the
423 European seafood industry. *Frontiers in Ecology and the Environment*, 8, 517–521

424

425 NOAA (National Oceanic and Atmospheric Administration). (2013). Imports and exports of fishery
426 products annual summary, <http://www.st.nmfs.noaa.gov/st1/publications.html> (accessed December
427 16, 2013).

428

429 Quinteiro J., Sotelo C. G., Rehbein H., Pryde S. E., Medina I., Pérez-Martín R. I., Rey-Méndez M., &
430 Mackie I. M. (1998). The use of mtDNA direct PCR-sequencing and PCR-RFLP methodologies in
431 species identification of canned tuna. *Journal of Agricultural and Food Chemistry*, 46, 1662-1669.

432

433 Rasmussen, R. S., & Morrissey, M. T. (2008). DNA-Based Methods for the Identification of
434 Commercial Fish and Seafood Species. *Comprehensive reviews in food science and food safety*, 7,
435 280-295.

436

437 Rasmussen, R. S., & Morrissey, M. T. (2009). Application of DNA-Based Methods to Identify Fish and
438 Seafood Substitution on the Commercial Market. *Comprehensive Reviews in Food Science and Food
439 Safety*, 8, 118-154.

440

441 Ratnasingham S., & Hebert P. D. N. (2007). BOLD: the barcode of life data system. *Molecular Ecology
442 Notes*, 7, 355–364.

443

444 Rehbein, H., & Oliveira, A. C. (2012). Alaskan flatfishes on the German market: part 1: identification
445 by DNA and protein analytical methods. *European Food Research and Technology*, 234, 245-251.

446

447 Sevilla, R. G., Diez, A., Norén, M., Mouchel, O., Jerome, M., Verrez-Bagnis, V., Van Pelt, H., Favre-
448 Krey, L., Krey, G., The Fishtrace Consortium & Bautista, J. M. (2007). Primers and polymerase chain
449 reaction conditions for DNA barcoding teleost fish based on the mitochondrial cytochrome b and
450 nuclear rhodopsin genes. *Molecular Ecology Notes*, 7, 730-734.

451

452 Schröder, U. (2008). Challenges in the traceability of seafood. *Journal of Consumer Protection and
453 Food Safety*, 3, 25-28.

454

455 Sotelo C. G., & Pérez-Martín R. I., (2007). Fish and Seafood Authentication. In S. E. Ebeler, G. R.
456 Takaoka, & P. Winterhalter (Eds.), *Authentication of Food and Wine* (pp.126-137). ACS Symposium
457 Series.

458

459 Teletchea, F. (2009). Molecular identification methods of fish species: reassessment and possible
460 applications. *Reviews in Fish Biology and Fisheries*, 19, 265-293.

461

462 Woolfe, M., Gurung, T., & Walker, M. J. (2013). Can Analytical Chemists do Molecular Biology? A
463 Survey of the Up-skilling of the UK Official Food Control System in DNA Food Authenticity
464 Techniques. *Food Control*, 33, 385-392.

465

466 Wong, E. H. K., & Hanner, R. H. (2008). DNA barcoding detects market substitution in North
467 American seafood. *Food Research International*, 41, 828–837.

468

469 Figure 1. PCA the species commonly tested in each authenticity laboratory. PC 1 incorporates 25.3%
470 and PC 2 18.9% of the variation (eigenvalues = 0.382 and 0.286, respectively).

471

472 Figure 2. PCA the methods utilised in each authenticity laboratory. PC 1 incorporates 42.5% and PC 2
473 27.4% of the variation (eigenvalues = 0.339 and 0.218, respectively). Methodological abbreviations;
474 FINS = forensically informative nucleotide sequencing, RFLP = restriction fragment length
475 polymorphism, IEF isoelectric focusing, rtPCR = real-time polymerase chain reaction & SSprimers =
476 species specific primers.

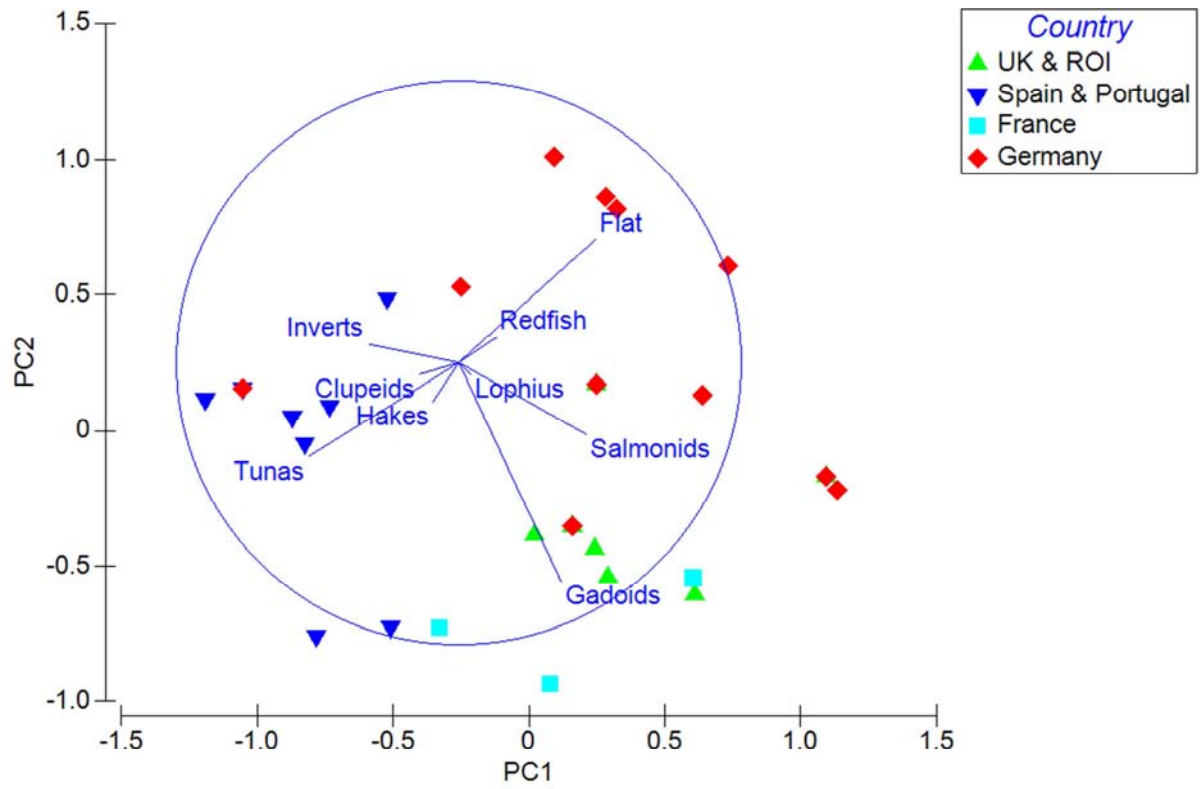
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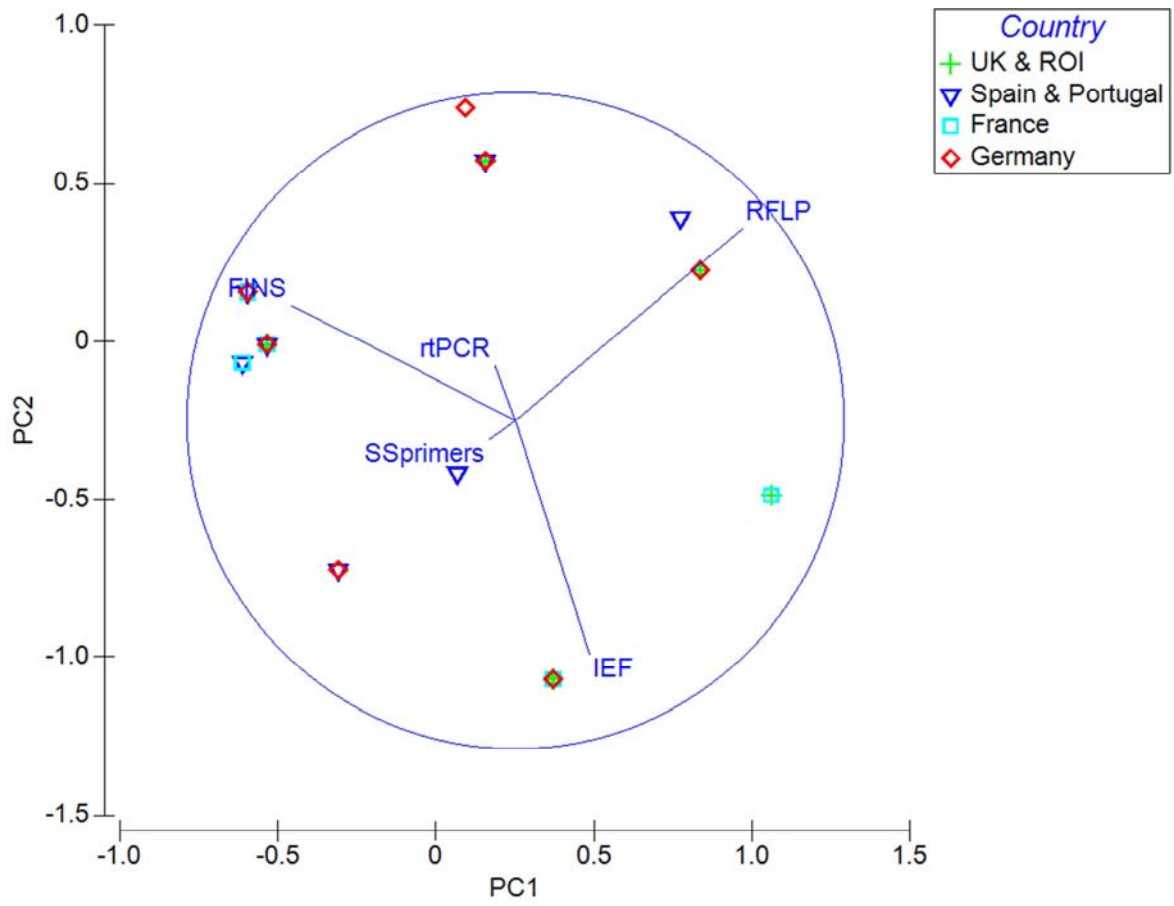
478 Table 1. Results of the ANOSIM testing for differences in responses among the countries surveyed.
 479 Both global and pair-wise tests between countries are included. Results significant at the 95%
 480 confidence interval are highlighted in bold, those remain significant after sequential Bonferroni
 481 correction (initial value 0.05/7) are also marked with *.

| Test | Species | | Methods | | DNA Markers | |
|--------------------------|--------------|---------------|--------------|---------------|--------------|---------------|
| | R statistic | p-value | R statistic | p-value | R statistic | p-value |
| Global | 0.447 | 0.001* | 0.259 | 0.001* | 0.121 | 0.050 |
| UK/ROI & Spain/Portugal | 0.633 | 0.091 | 0.362 | 0.001* | 0.320 | 0.002* |
| UK/ROI & France | 0.229 | 0.001* | 0.366 | 0.026 | 0.158 | 0.364 |
| UK/ROI & Germany | 0.377 | 0.035 | 0.358 | 0.001* | -0.034 | 0.675 |
| Spain/Portugal & France | 0.394 | 0.001* | 0.156 | 0.130 | 0.037 | 0.444 |
| Spain/Portugal & Germany | 0.526 | 0.001* | 0.041 | 0.200 | 0.180 | 0.026 |
| France & Germany | 0.276 | 0.062 | 0.383 | 0.011* | -0.005 | 0.505 |

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483





LabelFish is an EU effort to try and understand exactly what methods are currently being used for the identification of species in seafood. Once we have a better understanding, it is hoped that we can then propose more harmonised approaches that will allow investigation of mislabelling and traceability across Europe. Therefore, we ask for a little bit of information (nothing commercially sensitive, so please omit or be less specific on any questions you are unable to answer), to help us realise this objective.

These are our key questions:

- Are you a public or private testing facility?
- What biochemical or molecular method(s) do you use for distinguishing between species in the analysis of sea-foods?
- In any molecular genetic methods, what region of the DNA do you use (nuclear vs mitochondrial, specific protein coding genes or non-coding regions; can you tell us which you use for each taxonomic group)?
- What key pieces of equipment do you use in distinguishing between species?
- Are your methods universal or tailored to specific groups of fish?
- What species are you most commonly asked to check for mislabelling?
- Have you developed your own databases of reference material or baseline information to distinguish between species? How extensive are they?
- Are you developing any novel approaches that you could tell us about in broad terms?

The aim of LabelFish is not to develop new methods of species identification in the analysis of seafood, but find out how best to standardise the most effective approaches currently used across Europe. So, if you can give us any information it could help towards specific methods being employed more widely!

Thanks for any help you can provide!

| Country | Method | Universal or Specific | DNA region |
|----------|---|-----------------------|--|
| UK | Isoelectric focusing (Aglient/RFLP) | | |
| UK | FINS, RFLP/Agilent | Universal | Cyt b, COI |
| UK | RFLP/Agilent | Both | Cyt b, COI |
| UK | RFLP/Agilent | Universal | Cyt b |
| UK | RFLP | Both | Cyt b |
| UK | RFLP/Agilent, FINS | Both | Cyt b |
| UK | RFLP/Agilent | Both | Cyt b |
| UK | Isoelectric focusing (Aglient/RFLP) | Both | Cyt b |
| UK | Electrophoresis Phast system (Isoelectric focusing) | | |
| UK | RFLP/Agilent | Universal | Cyt b |
| Spain | FINS | both | mitochondrial (Cyt b, COI, 16S) |
| Spain | FINS, Real Time-PCR | both | mitochondrial (Cyt b, COI) |
| Spain | FINS, SDS-PAGE, IEF, RAPD | Universal | Nuclear (RAPD), mitochondrial (Cyt b) |
| Spain | FINS | Specific | Nuclear, mitochondrial |
| Spain | FINS | | mitochondrial (Control region) |
| Spain | FINS, Specific primers | both | mitochondrial (Cyt b, COI, 16S, control region) |
| Spain | Real Time-PCR, RFLP | universal | mitochondrial |
| Spain | SNPs, FINS, ELISA | universal | mitochondrial (COI, Cyt b) |
| Spain | FINS, RFLP | both | mitochondrial |
| Spain | FINS, RFLP | both | Nuclear and mitochondrial |
| Spain | multiplex PCR, Specific primers | both | Mitochondrial, nuclear (non co |
| France | specific PCR, FINS, DHPLC | both | mitochondrial (Cyt b) for FINS and DHPLC - confidential for specific PCR |
| France | FINS, next generation sequencing | Universal (patented) | mitochondrial |
| France | IEF, RFLP, | | Nuclear (pantophysin), mitochondrial (Cyt b) |
| France | IEF | | |
| France | PCR, RT-PCR, sequencing, clon | both | all depending on species or grou |
| ROI | FINS, microsatellite assignment | both | Cyt b, microsatellites |
| Portugal | FINS | both | 16S rDNA, Cyt b, COI |
| Germany | L 11.00-7, L 11.00-12 | universal | Cytb, |
| Germany | PCR, Realtime PCR, RFLP, Sequencing | universal /specific | Cytb, CytOx, 16SrRNA, Tmo-4C4, myostatin |
| Germany | L 11.00-12 | universal | mitochondrial DANN |

| | | | |
|---------|--|--|--|
| Germany | PCR-Sequencing | universal | Cytb, |
| Germany | L 11.00-12 | universal | Cytb, |
| Germany | L 11.00-6 | - | - |
| Germany | L 11.00-12 | universal | Cytb |
| Germany | L 11.00-12 | generally universal, sometimes specific | Cytb (§64-Method) Cytb for <i>Lates calcarifer</i> and <i>Lates niloticus</i> (Schiefenhövel & Rehbein, 2011), COI (Ivanova et al, 2007) 16S-rRNA (Pardo et al, 2005) |
| Germany | L 11.00-7 | universal | Cytb |
| Germany | L 11.00-7, L 11.00-12 | universal/specific | first choice: Cytb, nuclear parvalbumin, CytcOI, 16SrRNA, nuclear Calmodulin and Rhodopsin |
| Germany | only DNA- sequencing methods | generally universal, sometimes specific | mitochondrial and nuclear DNA |
| Germany | L 11.00-7, L 11.00-12 | universal | mitochondrial DNA |
| Germany | L 11.00-6, L 11.00-12 | universal | Cytb |
| Germany | L 11.00-7, L 11.00-12 | universal | Cytb |
| | L 11.00-6, L 10.00-12 | generally universal, sometimes specific | mitochondrial DNA : Cytb, CytOx, sometimes ATPase, NADH dehydrogenase or 16S rRNA |
| Germany | mostly Real Time PCR (Inhouse methods) , than PCR+ sequencing | specific Inhouse Real Time PCR methods | mitochondrial Gens |
| Germany | PCR + Sequencing | universal | Cytb |

| Species | databases | developing methods | Public or private insti |
|---|--------------------------|-----------------------------------|-------------------------|
| White fish | own | no | public |
| Gadoids, Salmonids, Pleuronectiformes, Tunas, Pangasius, Prawns | own, public | yes, novel agilent protocols | private |
| Salmoinds, White fish | own | no | public |
| White fish | own | no | public |
| Tuna, White fish, Hake, Pleuronectiformes | own | | private |
| White fish | own | FINS | public |
| White fish | both | no | public |
| Salmonids, White fish, Pleuronectiformes | own | no | public |
| White fish, salmonids, praen | own | no | private |
| Cod haddock | both | no | public |
| Tunas, Anchovies, gadoids | own, public | RT-PCR (TaqMan probes), PCR-ELISA | public |
| Tunas, Anchovies | own, public | TaqMan probes | private |
| | public | no | public |
| Bivalve molluscs | public | SSRs | public |
| Tunas, anchovies, bivalve molluscs | own, public | Real Time-PCR | public |
| hake, cod, tunas, anchovies and molluscs | own | Real Time-PCR | private |
| hake, sardine | public | no | private |
| hake, sardine, tuna | own | no | public |
| Tuna, cephalopods, mussels | own | Real Time-PCR | private |
| Mussels | public | RFLP | private |
| tunas | public | no | public |
| Theragra chalcogramma, cod, Ga | own, public | DHPLC | private |
| freshwater fish | own | | private |
| Gadus morhua, thunnus, lophius | own | no | public |
| cod, hoki, tuna, tropical soles, Ala | own | | private |
| ip species | clients's databases | no | private |
| salmonids | own | no | university |
| Fish, Crustacean, Mollusc | own, public | yes | private |
| sole | | | |
| butterfish | | | |
| salmonids | Fisch-DB | no | public |
| no special fish | own databases, EMBL/NCBI | no | private |
| a huge diversity of fish with main focus on sole | BLAST/NCBI | no | public |

| | | | |
|--|--|--|---------|
| Acipenser species (Caviar) | | | |
| Cod zander European perch | BLAST/NCBI | not in terms of fish | public |
| sole | BLAST/NCBI, own reference samples /sequences | no | public |
| plaice, turbot, flounder, sole | own reference sample | no | public |
| no special fish | NCBI | no | public |
| mostly: plaice, sole and halibut; regularly: gilthead seabream, saithe, cod, salmonids, pangasius in generell the hole range of fish species in the German market | BLAST/NCBI, own reference samples /sequences | no | public |
| saithe, Alaska pollack, cod, tilapia, pangasius, salmonids, sole, flounder, plaice, hake, redfish | PCR-RFLP : §64 method, FischDB, own reference samples | no | public |
| non specific | NCBI, barcode of live and Fish DB | no | public |
| the whole range of fishes, nowadays tuna, scallops and crustacea | own databank and NCBI | yes | private |
| salmonids and caviar | reference samples, §64-Method, Fish-DB for PCR- RFLP and NCBI for sequencing | no | public |
| sole, plaice, other flatfishes angler-fish, | own databank for IEF and Blast /NCBI | no | public |
| halibut, butterfish, tuna | NCBI | no | public |
| the whole range of fishes, main focus on flatfish, scallops, crustacean; nowadays snapper and anglerfish | NCBI, reference samples | no | public |
| Alaska pollack, tuna, hering, cod, pangasius, redfish, sardine, plaice, sole, turbot, halibut, tilapia | own databank for Real time PCR, BLAST/NCBI | yes: the species specific Real Time PCR methods | private |
| flatfish, other species | NCBI and Fishbase | yes | public |

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| Country | Species | Species Class | Gadoids/Pangasius | Salmonids |
|---------|---|---|-------------------|-----------|
| UK | White fish | Gadoids/Pangasius | 1 | 0 |
| UK | Gadoids, Salmonids, Pleuronectiformes, Tunas, Pangasius, Prawns | Gadoids/Pangasius, Salmonids, Pleuronectiformes, Tunas, Invertebrates | 1 | 1 |
| UK | Salmoinds, White fish | Gadoids/Pangasius, Salmonids | 1 | 1 |
| UK | White fish | Gadoids/Pangasius | 1 | 0 |
| UK | Tuna, White fish, Hake, Pleuronectiformes | Gadoids/Pangasius, Hakes, Pleuronectiformes, Tunas | 1 | 0 |
| UK | White fish | Gadoids/Pangasius | 1 | 0 |
| UK | White fish | Gadoids/Pangasius | 1 | 0 |
| UK | Salmonids, White fish, Pleuronectiformes | Gadoids/Pangasius, Salmonids, Pleuronectiformes | 1 | 1 |
| UK | White fish, salmonids, prawn | Gadoids/Pangasius, salmonids, Invertebrates | 1 | 1 |
| UK | Cod haddock | Gadoids/Pangasius | 1 | 0 |
| Spain | Tunas, Anchovies, gadoids | Tunas, Clupeids, Gadoids/Pangasius | 1 | 0 |
| Spain | Tunas, Anchovies | Tunas, Clupeids | 0 | 0 |
| Spain | Bivalve molluscs | Invertebrates | 0 | 0 |
| Spain | Tunas, anchovies, bivalve molluscs | Tunas, Invertebrates, Clupeids | 0 | 0 |
| Spain | hake, cod, tunas, anchovies and molluscs | Hakes, Tunas, Clupeids, Gadoids/Pangasius, Invertebrates | 1 | 0 |
| Spain | hake, sardine | Hakes, Tunas | 0 | 0 |
| Spain | hake, sardine, tuna | Hakes, Clupeids, Tunas | 0 | 0 |
| Spain | Tuna, cephalopods, mussels | Tunas, Invertebrates | 0 | 0 |
| Spain | Mussels | Invertebrates | 0 | 0 |
| Spain | tunas | Tunas | 0 | 0 |
| France | Theragra chalcogramma, cod, Gadus morhua, thunnus, lophius | Gadoids/Pangasius, Salmonids, Tuna | 1 | 1 |
| France | cod, hoki, tuna, tropical soles, Al | Gadoids/Pangasius, Lophius, Tunas | 1 | 0 |
| France | salmonids | Tuna, Gadoids/Pangasius, Pleuronectiformes | 1 | 1 |
| ROI | sole | salmonids | 0 | 1 |
| Germany | butterfish salmonids | Salmonids, Pleuronectiformes, Butterfish | 0 | 1 |
| Germany | a huge diversity of fish with main focus on sole | Pleuronectiformes | 0 | 0 |
| Germany | Acipenser species (Caviar) | | | |
| Germany | Cod zander | Caviar, Gadoids/Pangasius, Zander, Perch | 1 | 0 |
| Germany | European perch | | | |
| Germany | sole | Pleuronectiformes | 0 | 0 |
| Germany | plaice, turbot, flounder, sole | Pleuronectiformes | 0 | 0 |
| Germany | mostly: plaice, sole and halibut; regularly: gilthead seabream, saithe, cod, salmonids, pangasius in generell the hole range of fish species in the German market | Pleuronectiformes, Gadoids/Pangasius, salmonids, Seabream | 1 | 1 |

| | | | | |
|---------|--|---|-----------|-----------|
| Germany | saithe, Alaska pollack, cod, tilapia, pangasius, salmonids, sole, flounder, plaice, hake, redfish | Pleuronectiformes, Gadoids/Pangasius, Salmonids, Hakes, Redfish/Snapper | 1 | 1 |
| Germany | the whole range of fishes, nowadays tuna, scallops and crustacea | Tunas, Invertebrates | 0 | 0 |
| Germany | salmonids and caviar | Salmonids, Caviar | 0 | 1 |
| Germany | sole, plaice, other flatfishes angler-fish, | Pleuronectiformes, Lophius | 0 | 0 |
| Germany | halibut, butterfish, tuna | Pleuronectiformes, Tunas, Butterfish | 0 | 0 |
| Germany | the whole range of fishes, main focus on flatfish, scallops, crustacean; nowadays snapper and anglerfish | Pleuronectiformes, Lophius, Redfish/Snapper, Invertebrates | 0 | 0 |
| Germany | Alaska pollack, tuna, hering, cod, pangasius, redfish, sardine, plaice, sole, turbot, halibut, tilapia | Tuna, Gadoids/Pangasius, Clupeic | 1 | 0 |
| Germany | flatfish, other species | Pleuronectiformes | 0 | 0 |
| | | | 38 | 19 |
| | | | | 11 |

| | | | | | | | | |
|----|----|---|---|---|---|---|---|---|
| 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 15 | 15 | 5 | 9 | 4 | 2 | 2 | 1 | 1 |

| | | | | | | |
|----------|----------|----------|----------|----------|----------|----------|
| 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| 0 | 0 | 0 | 0 | 1 | 1 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | 1 | 1 | 1 | 3 | 1 | 1 |

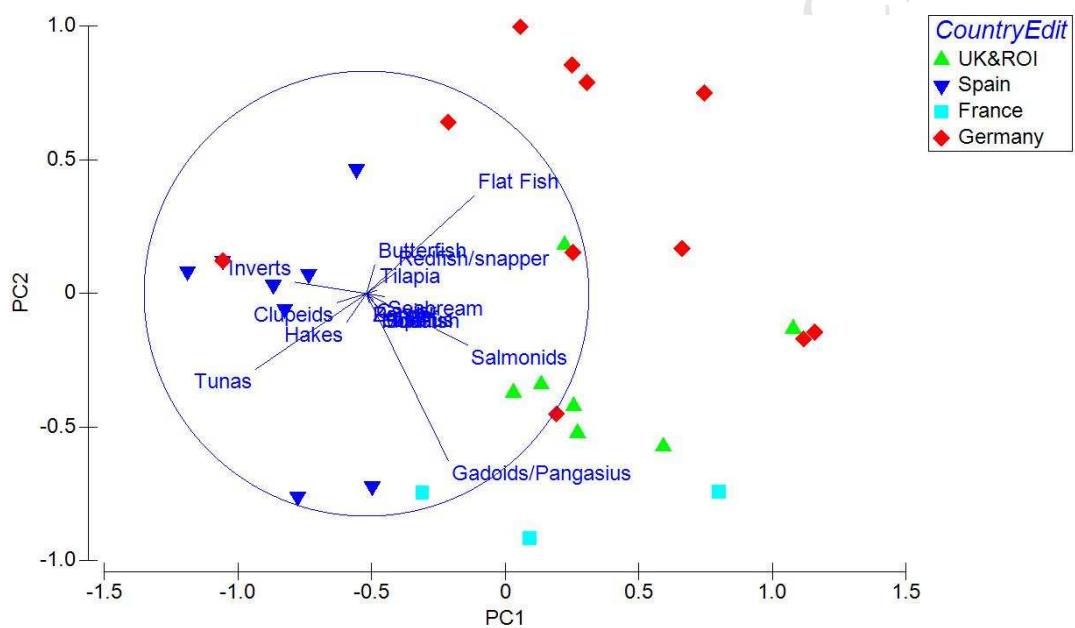


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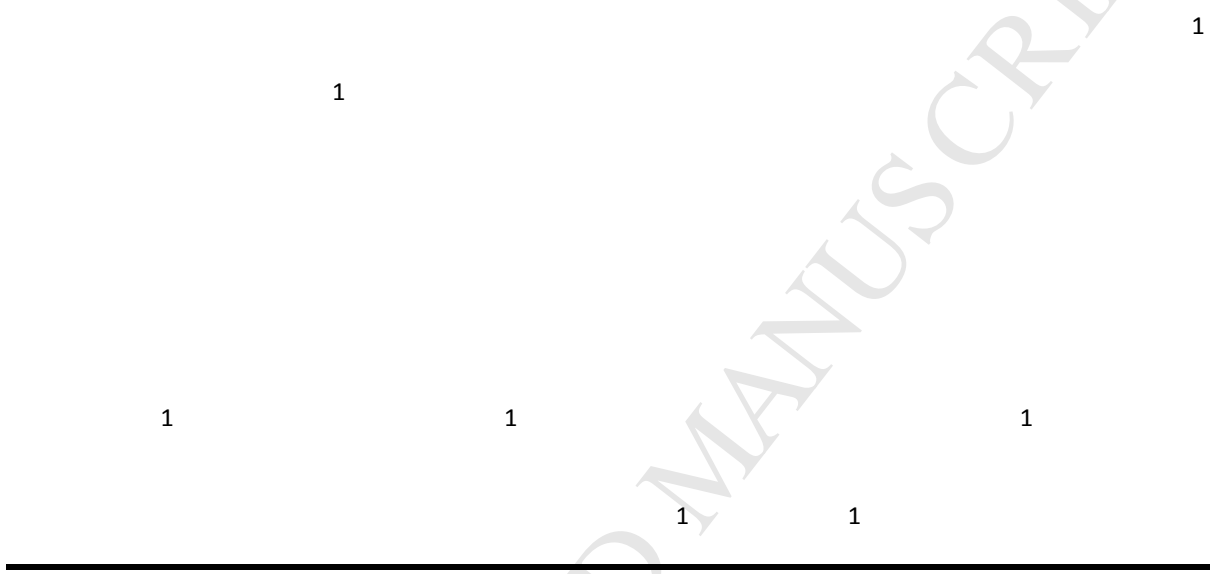
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Supplementary Material 4. PCA the species commonly tested in each authenticity laboratory, including all classifications of products (including the nine classes with only one or two responses that were removed from fig 1). PC 1 incorporates 21.6% and PC 2 16.4% of the variation (eigenvalues = 0.388 and 0.294, respectively).



| Country | Method classified | FINS | RFLP | IEF | rtPCR | Ssprimers |
|--------------|---------------------------|-----------|-----------|----------|----------|-----------|
| UK | IEF, RFLP | 0 | 1 | 1 | 1 | 0 |
| UK | FINS, RFLP | 1 | 1 | 1 | 0 | 0 |
| UK | RFLP | 0 | 1 | 1 | 0 | 0 |
| UK | RFLP | 0 | 1 | 1 | 0 | 0 |
| UK | RFLP | 0 | 1 | 1 | 0 | 0 |
| UK | FINS, RFLP | 1 | 1 | 1 | 0 | 0 |
| UK | RFLP | 0 | 1 | 1 | 0 | 0 |
| UK | IEF, RFLP | 0 | 1 | 1 | 1 | 0 |
| UK | IEF | 0 | 0 | 0 | 1 | 0 |
| UK | RFLP | 0 | 1 | 1 | 0 | 0 |
| Spain | FINS | 1 | 0 | 0 | 0 | 0 |
| Spain | FINS, rtPCR | 1 | 0 | 0 | 0 | 1 |
| Spain | FINS, SDS-PAGE, IEF, RAPD | 1 | 0 | 0 | 1 | 0 |
| Spain | FINS | 1 | 0 | 0 | 0 | 0 |
| Spain | FINS | 1 | 0 | 0 | 0 | 0 |
| Spain | FINS, Ssprimers | 1 | 0 | 0 | 0 | 0 |
| Spain | RFLP, rtPCR | 0 | 1 | 1 | 0 | 1 |
| Spain | SNPs, FINS, ELISA | 1 | 0 | 0 | 0 | 0 |
| Spain | FINS, RFLP | 1 | 1 | 1 | 0 | 0 |
| Spain | FINS, RFLP | 1 | 1 | 1 | 0 | 0 |
| Spain | Ssprimers | 0 | 0 | 0 | 0 | 0 |
| France | SSprimers, FINS, DHPLC | 1 | 0 | 0 | 0 | 0 |
| France | FINS, NGS | 1 | 0 | 0 | 0 | 0 |
| France | IEF, RFLP | 0 | 1 | 1 | 1 | 0 |
| France | IEF | 0 | 0 | 0 | 1 | 0 |
| France | RT-PCR, FINS | 1 | 0 | 0 | 0 | 1 |
| ROI | FINS, MSAT | 1 | 0 | 0 | 0 | 0 |
| Portugal | FINS | 1 | 0 | 0 | 0 | 0 |
| Germany | FINS, RFLP | 1 | 1 | 1 | 0 | 0 |
| Germany | FINS, RFLP, rtPCR | 1 | 1 | 1 | 0 | 1 |
| Germany | FINS | 1 | 0 | 0 | 0 | 0 |
| Germany | FINS | 1 | 0 | 0 | 0 | 0 |
| Germany | FINS | 1 | 0 | 0 | 0 | 0 |
| Germany | IEF | 0 | 0 | 0 | 1 | 0 |
| Germany | FINS | 1 | 0 | 0 | 0 | 0 |
| Germany | FINS | 1 | 0 | 0 | 0 | 0 |
| Germany | RFLP | 0 | 1 | 1 | 0 | 0 |
| Germany | FINS, RFLP | 1 | 1 | 1 | 0 | 0 |
| Germany | FINS | 1 | 0 | 0 | 0 | 0 |
| Germany | FINS, RFLP | 1 | 1 | 1 | 0 | 0 |
| Germany | IEF, FINS | 1 | 1 | 1 | 0 | 0 |
| Germany | IEF, FINS | 1 | 0 | 0 | 1 | 0 |
| Germany | IEF, FINS | 1 | 0 | 0 | 1 | 0 |
| Germany | rtPCR, FINS | 1 | 0 | 0 | 0 | 1 |
| Germany | FINS | 1 | 0 | 0 | 0 | 0 |
| TOTAL | | 31 | 19 | 9 | 9 | 3 |

rhodopsin pantophysin parvalbumin NADH ATPase Calmodulin non-coding rDNA



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Supplementary Material 6. PCA the methods utilised in each authenticity laboratory including all classifications of methods (including those with a single record were that were removed from fig 2). PC 1 incorporates 35.8% and PC 2 32.3% of the variation (eigenvalues = 0.341 and 0.221, respectively). Methodological abbreviations; FINS = forensically informative nucleotide sequencing, RFLP = restriction fragment length polymorphism, IEF isoelectric focusing, rtPCR = real-time polymerase chain reaction & SSprimers = species specific primers.

