1   Stable isotope signatures reveal small-scale spatial separation in populations of European sea
2   bass

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4   Giulia Cambiè[1], Michel J. Kaiser[1], Andrew L. Marriott[2], Jennifer Fox[1], Gwladys Lambert[1],
5   Jan G. Hiddink[1], Thomas Overy[1], Sarah A. Bennet[3], Melanie J. Leng[4] and Ian D. McCarthy[1]

6

7   [1] School of Ocean Sciences, Bangor University, Askew Street, Menai Bridge LL59 5AB, UK

8   [2] Inorganic Geochemistry Facility, Centre for Environmental Geochemistry, British
9   Geological Survey, Keyworth, Nottingham NG12 5GG, UK

10 [3]School of Life Sciences, Gibbet Hill Campus, The University of Warwick, Coventry, CV4
11 7AL, UK

12 [4] NERC Isotope Geosciences Laboratory, British Geological Survey, Keyworth, Nottingham
13 NG12 5GG, UK

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15 Running head: Isotopic signature in sea bass populations

16

17 ABSTRACT

18 Scientific information about European sea bass (*Dicentrarchus labrax*) stocks in NE Atlantic
19 is limited and a more accurate definition of the stock boundaries in the area is required to
20 improve assessment and management advice. Here we study the connectivity and movement
21 patterns of European sea bass in Wales (UK) using the stable isotope (δ^{13}C and δ^{15}N)
22 composition of their scales. Analysis of fish scale δ^{13}C and δ^{15}N values in the last growing
23 season was performed on 189 adult sea bass caught at nine coastal feeding grounds. Fish >50
24 cm total length (TL) caught in estuaries had very low δ^{13}C and this is characteristic of fresh
25 water (organic/soil) input, indicating the primary use of estuaries as feeding areas. A random
26 forest classification model was used to test if there was a difference in δ^{15}N and δ^{13}C values
27 between north, mid and south Wales and whether it was possible to correctly assign the fish
28 to the area where it was caught. This analysis was restricted to fish of a similar size range
29 (40-50 cm TL) caught in open coastal areas (n=156). The random forest classification model
30 showed that about 75% of the fish could be correctly assigned to their collection region based
31 on their isotope composition. The majority of the misclassifications of fish were fish from
32 north Wales classifying to mid Wales and vice versa, while the majority of fish from south
33 Wales were correctly assigned (80%). Our findings suggest that two sub-populations of sea

34 bass in Welsh waters use separate feeding grounds (south vs. mid/north Wales), and may
35 need separate management.

36

37 KEY WORDS: European sea bass, Stable isotopes, Random forest, Feeding ground, Stock
38 boundaries.

39

40   **INTRODUCTION**

41 Most current fisheries controls are applied at large geographic scales that often encompass

42 entire sea basins. Effort controls such as Minimum Landing Size (MLS) are only effective if

43 the life history and growth of fish is uniform across the scale at which they are applied.

44 However, for many species of fish, we have limited understanding of their home range and

45 hence the interaction between local environmental parameters and life history traits such as

46 growth rate potentially leading to a mismatch with the scale of management.

47 The European sea bass *(Dicentrarchus labrax)* is an economically important species

48 exploited by multiple fishing fleets across Europe (Pawson et al. 2007a, ICES 2013). In

49 northern Europe, the recent combination of declining recruitment and increasing fishing

50 mortality has led to a rapid decline in stock biomass and has triggered management advice for

51 an 80% reduction in catches as an immediate conservation measure (ICES 2014). A package

52 of emergency management measures was implemented in 2015 and then strengthened for

53 2016. For commercial fishing, these measures included a temporal ban on pelagic trawling

54 (six months) and on hooks, lines and fixed gill nets (two months), a monthly catch limit, an

55 increase in the MLS from 36 to 42 cm for northern sea bass and an area closure around

56 Ireland. For recreational fishers the measures include a six month moratorium followed by

57 one fish bag limit and the same increase of the MLS of the commercial sector

58 (http://ec.europa.eu/fisheries/cfp/fishing_rules/sea-bass/index_en.htm).

59 Although the stock structure of sea bass in the northeast Atlantic has not been clearly

60 delineated, there is evidence that sea bass around Ireland and in the Bay of Biscay could be

61 treated as two populations separate from the eastern Celtic Sea, English Channel, and North

62 Sea (Fritsch et al. 2007, ICES 2012). In addition, previous proposals of stock boundaries,

63 based on conventional tagging studies (ICES 2001, ICES 2002, ICES 2004, Fritsh et al. 2007,

64 Pawson et al. 2007b), concluded that there were two separate stock units between the east and

65 west UK (Pawson et al. 2007b). However, despite the inferences from tagging studies, ICES

66 has concluded that current evidence supports the view that sea bass in the North Sea (ICES

67 Division IVb&c) and in the Irish Sea, the English Channel and Celtic Sea (Ices Divisions

68 VIIa,d,e,f,g&h) should be treated as a functional stock unit as there is no clear basis at present

69 to subdivide them into independent stock units (ICES 2012). Clearly, a more accurate

70 definition of the stock boundaries is required to improve assessment and management advice

71 to underpin sustainable exploitation.

72 Previous tagging studies around England and Wales have shown a tendency for adult sea bass

73 to migrate to the south and west in autumn (English Channel), during the pre-spawning

74 season, and to return north and eastwards in spring to feeding areas (Pawson et al. 2007b,

75 Pawson et al. 2008). These mark-recapture studies have provided evidence of philopatry in

76 relation to feeding and spawning areas (Pawson et al. 2007b, Pawson et al. 2008). Pawson et

77 al. (2008) reported that 55% of sea bass > 40 cm that were tagged and released during the

78 summer were subsequently recaptured within 16 km of their original tagging location on their

79 summer feeding grounds. A further 23% were recaptured during winter at least 80 km from

80 their release site. These data provided strong evidence that sea bass in England and Wales

81 may share common migration routes to the same spawning grounds but may exhibit

82 segregation by returning to specific summer feeding grounds (Pawson et al. 2008). If this is

83 the case, then management of sea bass stocks may need to be applied at a much smaller

84 regional level than currently proposed by ICES.

85 Although the use of external tags can provide valuable information on stock structure and

86 movement patterns of fish (e.g. Dunn and Pawson 2002, Pawson et al. 2007b, Neuenfeldt et

87 al. 2013) there are multiple problems with their use that include, for example, transmission of

88 data to the researchers, and poor recapture rates (Block et al. 2011). Natural biogeochemical

89 markers (trace elements and stable isotopes) located in the hard parts of fishes (e.g otoliths

90 and scales) have great potential as 'internal' tags to study stock structure, and to study

91 connectivity between fish populations in, and movements between, chemically distinctive

92 water bodies by fishes during their lifetime (Elsdon et al. 2008, Trueman et al. 2012).

93 Structures such as otoliths and scales deposit new material incrementally as the fish grows,

94 and can provide a record of the elemental and isotopic composition of the water in which the

95 fish has lived at each stage of its lifetime (e.g. Thorrold et al. 1998, Cadrin et al. 2013).

96 The use of stable isotopes remains a relatively underused tool for tracking migration and

97 general movements in marine animals (Trueman et al. 2012). This is because there can be

98   uncertainties in the use of stable isotopes, in particular in the spatial distribution of stable-
99   isotope values across marine basins and in understanding patterns of isotope fractionation in
100   biological systems (Vander Zanden & Rasmussen 2001, Caut et al. 2009). In addition,
101   isotopic signatures vary temporally between body tissues that differ in their metabolic activity
102   and therefore represent an integration of feeding history over varying timescales from weeks
103   to months (Vander Zanden et al. 2015). However, incrementally growing hard tissue
104   structures such as scales and otoliths will embed within their structure an isotopic signature
105   laid down during a specific period of growth (specific timescale) (Rooker et al. 2008,
106   Sepulveda et al. 2009, Trueman et al. 2012). For fish scales in particular, the analysis of the
107   isotopic signature generally only refers to the most recent season of growth due to the
108   limitations imposed by scale architecture (Hutchinson & Trueman 2006). In fact, a typical sea
109   bass scale consists of two portions: a hard upper layer composed of calcium phosphate
110   overlying a poorly mineralised layer composed largely of collagen (Hutchinson & Trueman
111   2006). The collagenous layer grows by a process of underplating and for this reason only the
112   most recent season of growth is characterised by younger collagen (Hutchinson & Trueman
113   2006).

114   As our understanding of the spatial variation of isotope ratios in the aquatic environment has
115   developed, it has been possible to produce 'isoscapes' that map geographic changes in
116   aquatic isotopic signatures (see Graham et al. 2010, West et al. 2010). These isoscape maps
117   can then provide information on the movement patterns and foraging behaviour of study
118   species (see Graham et al. 2010, Hobson et al. 2010). For example, recent studies of scale
119   $\delta^{13}C$ and otolith $\delta^{13}C$ and $\delta^{18}O$ chemistry has provided valuable insights into the large-scale
120   marine migrations of Atlantic salmon and identification of their feeding areas at sea
121   (Mackenzie et al. 2011, Hanson et al. 2013).

122   The aims of this paper were to (1) measure the $\delta^{13}C$ and $\delta^{15}N$ composition in the last season
123   of growth in scales from adult sea bass caught at several locations around Wales (UK) and (2)
124   to use these isotopic signatures to infer patterns of movement and connectivity between
125   feeding areas to determine the possible presence of different stock units within Welsh coastal
126   waters. Spatial variation in $\delta^{15}N$ of the base of the foodweb for the Irish Sea has been
127   established with a 3‰ difference in $\delta^{15}N$ between north and south Wales (Jennings & Warr
128   2003). Therefore, if sea bass show philopatry to regional feeding grounds around the UK, as
129   suggested by Pawson et al. (2008), we predicted that sea bass caught in north and south

Wales would exhibit distinct scale isotopic signatures that could be used to classify fish back to summer feeding region.

MATERIALS AND METHODS

Data collection and preparation of samples

Scales from 189 adult sea bass (38.6-60.7 cm total length TL) were collected from a geographically representative range of coastal sites in Wales (Figure 1), 101 scales were collected during the feeding season (July-December 2013) and 88 during the spawning season (March-May 2014).

Due to the scale architecture, using collagen from the most recent growth period is the only way to obtain isotope data from the last (most recent) feeding season. Although sea bass scales are relatively large, the last season is often thin (depending on the month of capture, with an average width of section of 0.44 (±1.7 SD) mm), and therefore several scales from each individual fish were used to gather enough material (0.6 mg) for analysis. Individual scales were briefly soaked in Millipore™ ultra-pure water and manually cleansed using non-metallic forceps and a small nylon brush to remove any remaining adhering vestigial tissue. The last season of growth was trimmed from the top edge of the scale, weighed and placed into pre-weighed tin capsules. Decalcification of sea bass scales was not performed prior to isotopic analysis since the removal of inorganic carbonates has no significant effect on scale $\delta^{13}C$ and $\delta^{15}N$ values (Sinnatamby et al. 2007, Woodcock & Walther 2014).

Scale samples were analysed in a mass spectrometer. Analytical precision for $\delta^{13}C$ and $\delta^{15}N$ was based on both the long term reproducibility of calibrated in-house standards and repeat analysis of sample material. Standard deviation error for $\delta^{13}C$ was <0.1‰ and for $\delta^{15}N$ was <0.2‰.

Data analysis

Isotope ratios were expressed using a delta (δ) notation, representing parts per thousand (‰) deviations from the international standards PeeDee Belemnite (PDB) for carbon and air for nitrogen, according to the following equation:

160    $X = [(R_{sample} - R_{standard})/R_{standard}] \times 1000$ (1)

161   where X is $\delta^{13}C$ or $\delta^{15}N$ and R is the ratio of the heavy isotope to the light isotope ($^{13}C:^{12}C$ or

162    $^{15}N:^{14}N$) in the sample and the reference material.

163   We first measured the intra fish variability to determine whether the use of a single sample

164   per fish provided an accurate measurement of the isotopic signal. We tested seven fish (three

165   from north, one from mid and three from south Wales). For each fish, we prepared and

166   compared three replicates of scale material (last growing season) of 0.6 mg obtained from the

167   same single mix of scales collected from the body area under the pectoral fin. A repeated-

168   measures ANOVA was then applied to assess the presence of significant differences in the

169   isotopic signal between samples of the same fish.

170   Of the 189 fish whose samples were analysed in the present study, 28 (38.6-60.7 cm TL)

171   were caught in estuaries (Aberdovey-mid Wales, n=13 and Burry Port-south Wales, n=15)

172   while the remainder were caught in open coastal waters. A comparison of the $\delta^{13}C$ values

173   between the two groups (estuarine fish vs. marine fish) was undertaken using Welch's t-test.

174   This test was not performed for $\delta^{15}N$, due to the influence of the fish size on the accumulation

175   of the isotope ratio of this element (13 estuarine fish were > 50 cm TL) (Jennings et al. 2002).

176   To assess the possible differences in the isotope signal of sea bass collected during the

177   feeding and spawning season, sea bass from the same location (Hell's Mouth-Mid Wales, fish

178   provided by the same fisher and caught in the same spot, n=15 for both seasons) were

179   compared using a one-way ANOVA. This analysis explores how long sea bass stay in the

180   same feeding area. As the scale material is laid down during the feeding season, the scales

181   collected during the spawning season represent the isotopic signature of the area occupied by

182   the fish during the whole preceding feeding season and not only during part of it.

183   To assess the presence of spatial differences in the isotope composition between areas a

184   random forest classification model (R package "randomForest", Liaw & Weiner 2002) was

185   used. Random forest analysis (Breiman 2001) is a nonparametric technique derived from

186   classification and regression trees (CART). The decision-tree modelling approach requires

187   fewer assumptions than traditional parametric methods (e.g. Linear Discriminant Analysis)

188   (Strobl et al. 2009). In particular random forest analysis allows correlated predictor variables

189   to be utilised without transformation or exclusion to obtain unbiased predictions and

190   estimates of variable importance (Strobl et al. 2009). In this context, random forest analysis

191   has been shown to be preferred for discrimination based on otolith microchemistry when the

192   assumptions of the traditional parametric methods cannot be reached (Mercier et al. 2011).

193   The random forest model produces many classification trees from which are derived an

194   ensemble of classifications to predict the dependent variable (in our case "geographic

195   location" of sea bass) as a result of average assignment across trees (Strobl et al. 2007, Strobl

196   et al. 2009). By default, the random forest model partitions the data into 'training' (generally

197   70% of data) and 'test' samples selected at random from the data set. Whilst the training

198   samples are used to build the model, the test set is used to validate its performance.

199   Estuarine fish were excluded from this analysis, to remove the effect of the different salinities

200   encountered between freshwater and marine habitats on $\delta^{13}C$ (Doucett et al. 1999). The

201   analysis was also restricted to fish of a similar size range (40-50 cm TL) to remove the effect

202   of the fish size on the variation of $\delta^{15}N$ between individuals (e.g. Jennings et al. 2002). The

203   $\delta^{13}C$ and $\delta^{15}N$ values of 156 sea bass between 40 and 50 cm TL caught in coastal areas (98

204   caught during the feeding season and 58 during the spawning season) were then compared to

205   assess if spatial differences in isotope composition existed. The nine different capture

206   locations were first aggregated into three main groups: north, mid and south Wales (Figure

207   1). The random forest classification model was then used to test if there was a difference in

208   scale $\delta^{13}C$ and $\delta^{15}N$ values between north, mid and south Wales and whether it was possible

209   to correctly assign the fish to the area where it was caught based on scale isotopic signature.

210   In addition this statistical technique allowed the importance of each predictor variable in the

211   classification process to be evaluated and ultimately to identify specific isotopic signatures by

212   area. Based on the level of separation and/or overlap of the isotopic signatures between areas

213   we expected to derive insights on movement patterns related to feeding behaviour for adult

214   sea bass.

215   The association, or proximity, between each fish (characterized by the combination of $\delta^{13}C$

216   and $\delta^{15}N$), is the number of times that they occur together in the same terminal node. The

217   "randomForest" package normalized these counts to produce a proximity matrix that can be

218   analysed using a metric scaling method. The resultant Multi-Dimensional Scaling plot

219   represented the degree of differentiation in stable isotopes values between the three

220   geographic locations.

221   Conditional variable importance was reported to show the relative contribution of each

222   predictor variable ($\delta^{13}C$ and $\delta^{15}N$) to the classification performance. To evaluate the

223   conditional variable importance we measured the Mean Decrease Accuracy (MDA) of the

224 forest when the values of each predictor (δ^{13}C and δ^{15}N) are randomly excluded (or

225 permuted). The greater the decrease in the accuracy of the random forest resulting from the

226 exclusion (or permutation) of a single variable, the more important that variable is for

227 classification of the data.

228 To aid interpretation of the results of the random forest analysis, a conditional inference tree

229 was used. In particular this single-tree method helped to trace the effects of δ^{13}C and δ^{15}N on

230 the classification of the feeding locations of bass and ultimately to identify the range of δ^{13}C

231 and δ^{15}N values associated with each location. To this end, the "ctree" function for

232 conditional inference trees in the "party" R package (Hothorn et al. 2006) was used.

233 We finally assessed the degree of correlation between the average value of δ^{15}N for sea bass

234 scales for each location sampled in this study and the corresponding values of predicted

235 spatial variation in δ^{15}N baseline estimated from the isoscape map (Jennings & Warr 2003).

236 The model developed by Jennings & Warr (2003), which related the δ^{15}N of scallops of each

237 sampling site to day of sampling, shell height, depth, surface temperature, bottom

238 temperature and summer salinity was used to calculate the δ^{15}N baseline corresponding to our

239 capture locations. Since not all our sampling sites corresponded exactly to the scallop

240 samplings sites in Jennings & Warr (2003), we used the δ^{15}N baseline values corresponding

241 to the closest areas to our sampling sites (Colwyn Bay, site 28; Anglesey, site 27; Trefor, site

242 30; Hells Mouth, site 31; Aberdovey, site 33; Skokholm Island, site 34; Tenby, Burry Port

243 and Gower, site 37). Although this isoscape map was developed in 2002, the hydrodynamic

244 and biogeochemical processes controlling the distribution of carbon and nitrogen isotope

245 values are temporally stable (MacKenzie et al. 2014). All analyses were carried out using R

246 version 3.0.2 (R Core Team 2013).

247

248   **RESULTS**

249 The repeated measures ANOVA showed no significant difference between the three

250 replicates of the scale material in δ^{15}N ($F_{2,12}=0.33$, p=0.73) and δ^{13}C ($F_{2,12}=1.14$, p=0.35)

251 values (Table S1 in Supplement 1). This demonstrated that the use of a single sample per fish

252 provided an accurate measurement of the isotopic signal in scale material laid down during

253 the last feeding season.

254 No significant difference was found in the isotope data between the feeding and spawning

255 season for the fish caught in Hell's Mouth (North Wales) (One-way ANOVA, $F_{1,28}=0.17$,

256 p=0.68 for δ^{15}N; $F_{1,28}$=2.59, p=0.12 for δ^{13}C) (absolute values are reported in Table S2 in

257 Supplement 1). This result suggested that adult sea bass caught in the same area have similar

258 isotope composition even when caught at different times of the year.

259 The 28 sea bass caught in estuaries had a significantly lower δ^{13}C values (−18.22 ± 1.49‰)

260 than those caught in coastal areas (−13.31 ± 0.99‰) (Welch's t-test, t = 16.8, P < 0.001;

261 absolute values are reported in Table S3 in Supplement 1) which was expected given that

262 freshwater ecosystems generally have lower δ^{13}C values relative to marine systems (Doucett

263 et al. 1999). Out of these fish, 13 were adult sea bass > 50 cm TL.

264 Regional difference in the marine δ^{13}C and δ^{15}N values was detected (Table 1), such that

265 values from south Wales were separated from those of mid and north Wales (Figure 2a). The

266 two dimensional representation of the random forest analysis also showed the degree of

267 overlap of the isotopic signature between north and mid Wales (Figure 2b).

268 The random forest classification model built on the training samples had a discrimination

269 capacity c. 75%, which means that most fish were correctly classified between north, mid and

270 south Wales. For both the training (n=116) and test (n=40) data sets, the majority of the

271 misclassifications were fish from north Wales that were classified as mid Wales and vice

272 versa, while the majority of fish from south Wales were correctly assigned (80%) (Table 2).

273 In this case, the misclassification corresponded to regions (Tenby and Skokholm Island)

274 geographically closer to Mid Wales than the rest of the samples (Burry Port and Oxwich-

275 Gower).

276 The Mean Decrease Accuracy (MDA) of the forest showed that both variables (δ^{13}C and

277 δ^{15}N) were important for the classification process. On average, δ^{13}C seemed slightly more

278 important than δ^{15}N for classifying the fish. The importance of each isotope varied regionally

279 such that δ^{15}N was more important for classifying fish in north Wales, δ^{13}C for mid Wales

280 and both isotopes were important for classifying fish to south Wales (Table 3).

281 The most important primary split in the conditional inference tree was the δ^{13}C value; sea

282 bass from north and mid Wales were allocated on the branch corresponding to δ^{13}C≤−13.2‰

283 on the proportion of 82% (n=42) and 88% (n=30) respectively (Figure 3). Out of these

284 proportions, most of the fish from north Wales (83%, n=35) had a δ^{13}C value in the range of

285 −14.3‰ and −13.2‰ while fish from mid Wales equally split between the δ^{13}C value

286 comprised between −14.3‰ and −13.2‰ (47%, n=14) and δ^{13}C ≤−14.3‰. Only 13% (n=9)

287 of sea bass caught in south Wales was allocated on the branch corresponding to δ^{13}C≤−

288 13.2‰. In fact most of the sea bass from south Wales (87%) were characterized by $\delta^{13}C > -$
289 13.2‰. In particular 100% of fish with $\delta^{13}C > -13.2$‰ and $\delta^{15}N \leq 15.47$‰ were from south
290 Wales. This specific signature characterised 68% of the total samples from south Wales
291 (Figure 3).

292 A strong linear relationship was found between the average values of scale $\delta^{15}N$ measured in
293 this study by location (y) and the corresponding values of the $\delta^{15}N$ baseline in the vicinity of
294 the sampling location (from Jennings & Warr 2003) (x) (y=0.674x + 9.83, P<0.001, adjusted
295 $R^2 = 0.81$) (Figure 4). This demonstrates that the spatial differences in $\delta^{15}N$ detected in this
296 study are consistent with the spatial patterns in $\delta^{15}N$ that occur at the base of the food web
297 suggesting localised feeding in Welsh sea bass.

298

299 **DISCUSSION**

300 The use of isotope tags to study the movement of fish by linking the stable isotope
301 composition measured in fish tissue to that of the base of the food chain at the site of origin is
302 a powerful tool that can help to better define stock boundaries at a local level (Trueman et al.
303 2012). The $\delta^{13}C$ and $\delta^{15}N$ values of tissue protein are controlled by the trophic level of the
304 fish and $\delta^{13}C$ and $\delta^{15}N$ values for primary production at the location occupied by the fish
305 during periods of active growth (e.g. Deniro & Epstein 1978, Fry 1981). The highly
306 significant relationship between the average value of $\delta^{15}N$ found in fish scales and the
307 average value of $\delta^{15}N$ baseline by location (Jennings & Warr 2003) confirmed our
308 assumption that the observed spatial differences in sea bass $\delta^{15}N$ reflected spatial differences
309 in $\delta^{15}N$ of the base of the food web rather than differences in sea bass feeding strategies. In
310 this context the use of the last growing season of sea bass scales can represent an advantage
311 in the analysis because the corresponding isotopic signature takes into account the temporal
312 variability in prey type and abundance across whole of the previous feeding season. Our
313 results thus suggest that much of the spatial variance in the $\delta^{15}N$ of sea bass along the Welsh
314 coast could be attributed to differences in base $\delta^{15}N$ and not in the diet composition, which
315 could be considered, to a first approximation, similar across Wales. Similar results have been
316 obtained for other predatory fishes such as dab *Limanda limanda* and whiting *Merlangius*
317 *merlangus*, for which the spatial variance in $\delta^{15}N$ was mostly attributed to differences in $\delta^{15}N$
318 at the base of the food chain (Jennings & Warr 2003).

319   Our results also showed that the average $\delta^{15}N$ in fish scales tended to increase with latitude.

320   This south-north gradient of $\delta^{15}N$ seems to be confirmed from other studies, which

321   documented higher values of $\delta^{15}N$ associated with cooler temperatures (Jennings et al. 2008)

322   and higher levels of oxygen (Radabaugh et al. 2013). While the mechanisms underpinning the

323   spatial isotopic gradients are not fully understood and several factors may influence the

324   spatial variation in $\delta^{15}N$ values, the degree of resuspension of particulate organic nitrogen is

325   likely to be a major factor (e.g. Saino & Hattori 1987). Aberdovey (mid Wales) was the only

326   location where sea bass had lower $\delta^{15}N$ compared to two areas further south. It is possible

327   that the sea bass from Aberdovey (a site at the mouth of a large estuary) were caught along a

328   freshwater-marine gradient, a fact that could explain this apparent discrepancy, as terrestrial

329   inputs to freshwater bodies are more $\delta^{15}N$-depleted than marine plankton (Schoeninger &

330   DeNiro 1984, Owens 1987). This interpretation would also be consistent with lower $\delta^{13}C$

331   with respect to fish from the other coastal areas, which could confirm that sea bass caught in

332   Aberdovey originated from a feeding area with freshwater influence (Doucett et al. 1999).

333   Our study also demonstrates that the random forest analysis, although not yet widely used in

334   marine ecology for hierarchical classification (e.g Mercier et al. 2011), is a powerful

335   statistical tool, as it allows not only predictors to be used without transformation or exclusion

336   but also the relative importance of the variables in the classification process to be estimated

337   (Strobl et al. 2009). Therefore, while $\delta^{15}N$ appeared more important than $\delta^{13}C$ in classifying

338   adult sea bass in north Wales (c.f. fish from mid Wales), both variables were necessary to

339   correctly classify fish from south Wales. Moreover the conditional inference tree allowed us

340   to identify the range of $\delta^{13}C$ and $\delta^{15}N$ values associated with each location and thus to

341   establish a geographic range of the isotopic signatures. Additional variables (e.g. element

342   concentration, Sr, Ba, Mn and Mg) could be used in the future to improve the discriminatory

343   power of the random forest and thus the precision level of the biogeochemical tag (Wells et

344   al. 2000, Ramsay et al. 2011, Seeley et.al 2015).

345   Our results showed that the stable isotope composition of fish scales provides unique insights

346   into fish movement at a regional scale (e.g. the Welsh coastline) without the need for

347   expensive conventional tagging studies. While fine-scale ontogenetic changes in habitat use

348   have been identified for other fish species (e.g. NE Atlantic orange roughly, Shephard et al.

349   (2007)), the use of fish scales has been limited to few species to date (Ramsay et al. 2012;

350   Woodcock & Walther 2014). The analysis of $\delta^{13}C$ and $\delta^{15}N$ in adult sea bass collected around

351   Welsh waters showed a high degree of overlap in the isotope composition between north and

mid Wales. This finding could be explained by ecological patterns of sea bass population in mid and north Wales and/or by abiotic factors such as temperature in these areas. While the overlapping of the isotopic signature in sea bass scales could indicate the presence of connectivity between north and mid Wales for feeding behaviour, it might also depend on a similar temperature range in these areas, as the isotopic signature is related to productivity, which is temperature-driven (e.g Fogel & Cifuentes 1993). Fish from south Wales appeared more isolated and characterised by a very distinctive isotopic signature, especially fish from the most southern areas (Burry Port and Oxwich-Gower). This finding suggests the possible presence of (at least) two separated sub-populations of sea bass in Welsh waters with little mixing between sea bass in the south with those in mid and north Wales.

This separation is based on fish locations during the feeding season, and thus our study confirms that adult sea bass do spend extended periods of time feeding in regional inshore areas as suggested by Pawson et al. (2007b and 2008). In this sense, the lack of difference in the isotopic signature of sea bass caught in Hell's Mouth-mid Wales (the only area where sea bass were caught in the same exact location) between feeding and spawning season, support the previous evidence that adult fish spend most of the feeding season in the same location. In fact the scale material is laid down during the feeding season and therefore the scales collected during the spawning season represent the isotopic signature of the area occupied by the fish during the whole preceding feeding season. Similar fidelity has been demonstrated, through tagging experiments, for other species in the region, such as plaice *Pleuronectes platessa*, which remained on the same feeding grounds throughout the summer and autumn (Dunn & Pawson 2002). In addition, stock boundaries were defined at a regional level with restricted stock units in the north-east Irish Sea, the western Irish Sea, and a stock in the south-east Irish Sea (Dunn & Pawson 2002).

No information on the presence of local separate spawning areas is currently available. Due to this lack of information, three possible scenarios remain open: 1) the two sub-populations mix during the spawning season and are part of the same stock, 2) they do not mix during the spawning season and can be therefore considered as two separate sub-populations, 3) they mix only partially. Future research should address this topic and the isotopic signatures identified in this study could be used to track the origin of individuals from spawning aggregations. However, whichever scenario is correct, it is already clear that different spatial scales of movement characterize the two sub-populations. Adult sea bass from south Wales are likely to have a restricted range of movement, not only for feeding behaviour as

385 demonstrated by this study but also for spawning, considering the proximity of a well-known

386 spawning ground of the species outside the Bristol Channel (known as "Trevose Head",

387 Lancaster et al. 1998, Reynolds et al. 2003). In this sense, the south Wales sub-population

388 would have a "resident" behaviour, where feeding grounds and spawning grounds are all

389 within close geographical proximity to one another. This trait has been found in many

390 populations of the Atlantic cod *Gadus morhua* where the residence areas (nursery, feeding

391 and spawning grounds) were geographically close and had relatively stable environmental

392 conditions (Robichaud & Rose 2004). In contrast, adult sea bass from mid and north Wales

393 either undertake a long-distance migration to the Trevose Head spawning area and/or other

394 potential spawning grounds in south Wales/England or, alternatively, a small-scale migration

395 to possible spawning grounds located off the coast of mid and/or north Wales. Our results

396 provide for the first time insights into a regional segregation of the sea bass stock, which

397 implies that local/regional management may be more appropriate than the present single

398 stock approach. In this context, specific regulations based on regional variation in the ecology

399 and life history of sea bass could facilitate a more rapid achievement of the conservation

400 objectives.

401 Our results also suggest that a portion of the largest sea bass adopt estuaries as preferential

402 feeding areas. In fact all fish with a total length >50 cm caught in estuarine areas showed very

403 low δ^{13}C, indicating the primary use of estuaries as feeding areas, as freshwater ecosystems

404 are generally δ^{13}C depleted relative to marine systems (Doucett et al. 1999). It is possible that

405 interspecific competition for resources has resulted in some big adult sea bass adopting an

406 estuarine feeding habit. Estuaries have been recognised to support the early phase of

407 development of the species (Kelley 1988, Lancaster et al. 1998) as well as part of the juvenile

408 stock (3-5 years old) (Pawson et al. 2005) and the present study stressed the importance of

409 this habitat for large adult sea bass. While other fish species, such as flathead mullet *Mugil*

410 *cephalus*, have been recognised as using estuarine areas through the juvenile and/or adult life

411 stages (Elliott et al. 2007), few previous studies have mentioned the presence of adult sea

412 bass in estuarine habitats in NE Atlantic (Kelley 1987, Kennedy & Fitzmaurice 1972, Pickett

413 & Pawson 1994). Moreover, these studies focused on describing the opportunistic feeding

414 behaviour of the species and its different habitat use, without associating estuaries with a

415 specific ecological role in the adult phase. Our results indicated estuarine feeding of some

416 large sea bass during extended time periods, possibly during the whole feeding season.

13

Estuarine areas could therefore be characterised by higher protection levels, if protecting large sea bass (e.g. large spawners) is a management target.

In conclusion, the use of the natural variability of the stable isotope composition of fish scales to describe movement patterns of a migratory fish species is an emerging area of research (e.g. MacKenzie et al. 2011, Ramsay et al. 2012, Woodcock & Walther 2014). This technique has useful applications to identify the use of different feeding grounds by individuals from the same population, and also offers a non-lethal alternative to the use of otoliths to determine geographic segregation and/or connectivity between foraging areas. Combined δ^{13}C and δ^{15}N measurements of fish scales can clearly identify regional feeding areas providing insights on the feeding ecology at local level and contributing to a better definition of the stock structure and the related management units.

LITERATURE CITED

Block BA, Jonsen ID, Jorgensen SJ, Winship AJ, Shaffer SA, Bograd SJ, Hazen EL, Foley DG, Breed GA, Harrison AL, Ganong JE, Swithenbank A, Castleton M, Dewar H, Mate BR, Shillinger GL, Schaefer KM, Benson SR, Weise MJ, Henry RW, Costa DP (2011) Tracking apex marine predator movements in a dynamic ocean. Nature 475:86–90

Breiman, L (2001) Random Forest. Mach Learn 45:5–32

Cadrin S, Kerr LA, Mariani S (2013) Stock Identification Methods 2nd edition. Academic Press.

447   Caut S, Angulo E, Courchamp F (2009) Variation in discrimination factors (Delta N-15 and

448   Delta C-13): the effect of diet isotopic values and applications for diet reconstruction. J App

449   Ecol 46:443–453

450   Deniro MJ, Epstein S (1978) Influence of diet on distribution of carbon isotopes in animals.

451   Geochim Cosmochim Acta 42:495–506

452   Doucett RR, Hooper W, Power G (1999) Identification of anadromous and nonanadromous

453   adult brook trout and their progeny in the Tabusintac River, New Brunswick, by means of

454   multiple-stable-isotope analysis. Trans Am Fish Soc 128:278–288

455   Dunn MR, Pawson MG (2002). The stock structure and migrations of plaice populations on

456   the west coast of England and Wales. J Fish Biol 61:360–393

457   Elliott M, Whitfield AK, Potter IC, Blaber SJM, Cyrus DP, Nordlie FG, Harrison TD (2007)

458   The guild approach to categorizing estuarine fish assemblages: a global review. Fish Fish

459   8:241–268

460   Elsdon TS, Wells BK, Campana SE, Gillanders BM, Jones CM, Limburg KE, Secor DH,

461   Thorrold SR, Walther BD (2008) Otolith chemistry to describe movements and life-history

462   parameters of fishes: Hypotheses, assumptions, limitations and inferences. Oceanogr Mar

463   Biol 46:297–330

464   Fogel ML, Cifuentes LA (1993) Isotope fractionation during primary production. In: Engel

465   MH, Macko SA (eds) Organic Geochemistry: Principles and Applications. Plenum Press,

466   New York; p 73–98

467   Fritsch M, Morizur Y, Lambert E, Bonhomme F, Guinand B (2007) Assessment of sea bass

468   (*Dicentrarchus labrax*, L.) stock delimitation in the Bay. Fish Res 83:123–132

469   Fry B (1981) Natural stable carbon isotope tag traces Texas shrimp migrations. Fish Bull

470   79:337–345

471   Graham BS, Koch PL, Newsome SD, McMahon KW, Aurioles D (2010) Using isoscapes to

472   trace the movements and foraging behavior of top predators in oceanic ecosystems. In: (West

473   JB, Bowen GJ, Dawson TE, Tu KP (eds) Isoscapes: Understanding Movement, Pattern, And

474   Process On Earth Through Isotope Mapping. Springer, New York; p 299–318

475   Hanson NN, Wurster CM, Todd CD (2013) Reconstructing marine life-history strategies of
476   wild Atlantic salmon from the stable isotope composition of otoliths. Mar Ecol Prog Ser
477   475:249–266

478   Hobson KA, Barnett-Johnson R, Cerling T (2010) Using isoscapes to track animal migration.
479   In: West JB, Bowen GJ, Dawson TE, Tu KP (eds) Isoscapes: Understanding Movement,
480   Pattern, And Process On Earth Through Isotope Mapping. Springer, New York; p 273–298

481   Hothorn T, Hornik K, Zeileis A (2006) Unbiased Recursive Partitioning: A Conditional
482   Inference Framework. J Comput Graph Stat 15:651–674

483   Hutchinson JJ, Trueman CN (2006) Stable isotope analyses of collagen in fish scales:
484   limitations set by scale architecture. J Fish Biol 69:1874–1880

485   ICES (2001) Report on the ICES Study Group on bass. CM 2001/ACFM:25

486   ICES (2002) Report on the ICES Study Group on bass. CM 2002/ACFM:11 ref.G

487   ICES (2004) Report of the Study Group on Bass, Lowestoft, England, August 2003. ICES
488   Document, CM 2004/ACFM: 04

489   ICES (2012) Report of the Inter-Benchmark Protocol on New Species (Turbot and Sea bass;
490   IBPNew 2012), 1–5 October 2012, Copenhagen, Denmark. ICES CM 2012/ACOM:45

491   ICES (2013) Report of the Working Group for Celtic Seas Ecoregion (WGCSE), 8–17 May
492   2013, Copenhagen, Denmark. ICES CM 2013/ACOM:12

493   ICES (2014) European sea bass in Divisions IVbc, VIIa, and VIId–h (Irish Sea, Celtic Sea,
494   English Channel, and southern North Sea). ICES Advice 2014, Book 5

495   Jennings S, Barnes C, Sweeting CJ, Polunin NVC (2008) Application of nitrogen stable
496   isotope analysis in size-based marine food web and macroecological research. Rapid
497   Commun Mass Spectrom 22:1673–1680

498   Jennings S, Pinnegar JK, Polunin NVC, Warr KJ (2002) Linking size-based and trophic
499   analyses of benthic community structure. Mar Ecol Prog Ser 226:77–85

500   Jennings S, Warr KJ (2003) Environmental correlates of large-scale spatial variation in the
501    $\delta^{15}N$ of marine animals. Mar Biol 142:1131–1140

502   Kelley DF (1987) Food of bass in U.K. waters. J Mar Biol Assoc UK 67:275–286

503 Kelley DF (1988) The importance of estuaries for sea bass *Dicentrarchus Labrax* (L.). J Fish
504 Biol 33 (Suppl. A):25–33

505 Kennedy M, Fitzmaurice P (1972) The biology of the bass, *Dicentrarchus labrax*, in Irish
506 Waters. J Mar Biol Assoc UK 52:557–597

507 Lancaster JE, Pawson MG, Pickett GD, Jennings S (1998) The Impact of the "Sea Empress"
508 oil spill on seabass recruitment. Mar Pollut Bull 36:677–688

509 Liaw A, Wiener M (2002) Classification and regression by randomForest. R News 2/3:18–22

510 MacKenzie K, Longmore C, Preece C, Lucas C, Trueman C (2014) Testing the long-term
511 stability of marine isoscapes in shelf seas using jellyfish tissues. Biogeochemistry 121:441–
512 454

513 MacKenzie KM, Palmer MR, Moore A, Ibbotson AT, Beaumont WRC, Poulter DJS,
514 Trueman CN (2011) Locations of marine animals revealed by carbon isotopes. Sci Rep 1:21

515 Mercier L, Darnaude AM, Bruguier O, Vasconcelos RP, Cabral HN, Costa MJ, Lara M,
516 Jones DL, Mouillot D (2011) Selecting statistical models and vari- able combinations for
517 optimal classification using otolith microchemistry. Ecol Appl 21:1352–1364

518 Neuenfeldt S, Righton D, Neat F, Wright PJ, Svedang H, Michalsen K, Subbey S, Steingrund
519 P, Thorsteinsson V, Pampoulie C, Andersen KH, Pedersen MW, Metcalfe J (2013) Analysing
520 migrations of Atlantic cod Gadus morhua in the north-east Atlantic Ocean: then, now and the
521 future. J Fish Biol 82:741–763

522 Owens NJP (1987) Natural variations in I5N in the marine environment. Adv Mar Biol
523 24:389–451

524 Pawson MG, Brown M, Leballeur J, Pickett GD (2008) Will philopatry in sea bass,
525 Dicentrarchus labrax, facilitate the use of catch-restricted areas for management of
526 recreational fisheries? Fish Res 93:240–243

527 Pawson MG, Kupschus S, Pickett GD (2007a) The status of sea bass (Dicentrarchus labrax)
528 stocks around England and Wales, derived using a separable catch-at-age model, and
529 implications for fisheries management. ICES J Mar Sci 64:346–356

530   Pawson MG, Pickett GD, Leballeur J, Brown M, Fritsch M (2007b) Migrations, fishery

531   interactions and management units of sea bass (*Dicentrarchus labrax*) in Northwest Europe.

532   ICES J Mar Sci 64:332–345

533   Pawson MG, Pickett GD, Smith MT (2005) The role of technical measures in the recovery of

534   the UK sea bass (*Dicentrarchus labrax*) fishery 1980–2002. Fish Res 76:91–105

535   Pickett GD, Pawson MG, 1994. Sea bass. Biology, exploitation and conservation. Chapman

536   & Hall, London

537   R Core Team (2013) R: a language and environment for statistical computing. R Foundation

538   for Statistical Computing, Vienna, Austria. http://www.R-project.org/

539   Radabaugh KR, Hollander DJ, Peebles EB (2013) Seasonal δ^{13}C and δ^{15}N isoscapes of fish

540   populations along a continental shelf trophic gradient. Cont Shelf Res 68:112–122

541   Ramsay AL, Milner NJ, Hughes RN, McCarthy ID (2011) Comparison of the performance of

542   scale and otolith microchemistry as fisheries research tools in a small upland catchment. Can

543   J Fish Aquat Sci 68:1–11

544   Ramsay AL, Milner NJ, Hughes RN, McCarthy ID (2012) Fish scale δ^{15}N and δ^{13}C values

545   provide biogeochemical tags of fish comparable in performance to element concentrations in

546   scales and otoliths. Hydrobiologia 694:183–196

547   Reynolds WJ, Lancaster JE, Pawson MG (2003) Patterns of spawning and recruitment of

548   bass to Bristol Channel nurseries in relation to the 1996 "Sea Empress" oil spill. J Mar Biol

549   Assoc UK 83:1163–1170

550   Robichaud D, Rose GA (2004) Migratory behaviour and range in Atlantic cod: inference

551   from a century of tagging. Fish Fish 5:185–214

552   Rooker JR, Secor DH, DeMetrio G, Kaufman AJ, Rios AB, Ticina V (2008) Evidence of

553   trans-Atlantic movement and natal homing of bluefin tuna from stable isotopes in otoliths.

554   Mar Ecol Prog Ser 368:231–239

555   Saino T, Hattori A (1987) Geographical variation of the water column distribution of

556   suspended particulate organic nitrogen and its ^{15}N natural abundance in the Pacific and its

557   marginal seas. Deep-Sea Res 34:807–827

558 Schoeninger MJ, DeNiro MJ (1984) Nitrogen and carbon isotopic composition of bone
559 collagen from marine and terrestrial animals Geochim Cosmochim Acta 48:625–639

560 Seeley M, Miller N, Walther B (2015) High resolution profiles of elements in Atlantic tarpon
561 (Megalops atlanticus) scales obtained via cross-sectioning and laser ablation ICP-MS: a
562 literature survey and novel approach for scale analyses. Environ Biol Fish 98:2223–2238

563 Sepulveda AJ, Colyer WT, Lowe WH, Vinson MR (2009) Using nitrogen stable isotopes to
564 detect long-distance movement in threatened cutthroat trout (*Oncorhynchus clarkii* utah). Can
565 J Fish Aquat Sci 66:672–682

566 Shephard S, Trueman C, Rickaby R, Rogan E (2007) Juvenile life history of NE Atlantic
567 orange roughy from otolith stable isotopes. Deep Sea Res Part 1 Oceanogr Res Pap 54:1221–
568 1230

569 Strobl C, Boulesteix AL, Zeileis A, Hothorn T (2007) Bias in random forest variable
570 importance measures: illustrations, sources and a solution. BMC Bioinformatics 8:25

571 Strobl C, Malley J, Tutz G (2009) An introduction to recursive partitioning: rationale,
572 application, and characteristics of classification and regression trees, bagging, and random
573 forests. Psychol Methods 14:323–348

574 Thorrold SR, Jones CM, Swart PK, Targett TE (1998) Accurate classification of juvenile
575 weakfish *Cynoscion regalis* to estuarine nursery areas based on chemical signatures in
576 otoliths. Mar Ecol Prog Ser 173:253–265

577 Trueman CN, MacKenzie KM, Palmer MR (2012) Identifying migrations in marine fishes
578 through stable-isotope analysis. J Fish Biol 81:826–847

579 Vander Zanden MJ, Clayton MK, Moody EK, Solomon CT, Weidel BC (2015) Stable
580 isotope turnover and half-life in animal tissues: a literature synthesis. PLoS ONE
581 10(1):e0116182

582 Vander Zanden MJ, Rasmussen JB (2001) Variation in $\delta^{15}N$ and $\delta^{13}C$ trophic fractionation:
583 Implications for aquatic food web studies. Limnol Oceanogr 46:2061–2066

584 Wells BK, Thorrold SR, Jones CM (2000) Geographic variation in elemental signatures of
585 weakfish scales. T Am Fish Soc 129:889–900.

586 West JB, Bowen GJ, Dawson TE, Tu KP (2010) Isoscapes: Understanding Movement,
587 Pattern, And Process On Earth Through Isotope Mapping. Springer, New York

588 Woodcock SH, Walther BD (2014) Trace elements and stable isotopes in Atlantic tarpon
589 scales reveal movements across estuarine gradients. Fish Res 153:9–17

590

591   Table 1. Values (mean ± SD) of $\delta^{13}C$ and $\delta^{15}N$ in scale material formed during the last

592   growth period of the adult sea bass *Dicentrarchus labrax* (40-50 cm TL, n=156) caught in

593   coastal areas around Wales.

Area	Location	$\delta^{13}C$ (VPDB)‰	$\delta^{15}N$ (AIR)‰
North Wales (n=51)	Colwyn Bay	−13.85 ± 0.61	16.76 ± 1.06
	Anglesey	−13.35 ± 0.69	15.96 ± 0.50
	Trefor	−13.62 ± 0.61	16.06 ± 1.40
Mid Wales (n=34)	Hells Mouth	−14.02 ± 0.70	15.64 ± 1.09
	Aberdovey	−15.05 ± 0.61	14.84 ± 0.39
South Wales (n=71)	Skokholm Island	−12.98 ± 1.20	15.43 ± 0.79
	Tenby	−12.45 ± 0.50	14.82 ± 0.62
	Burry Port	−12.78 ± 0.68	15.24 ± 1.27
	Gower	−12.36 ± 0.48	14.52 ± 0.70

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595

596 Table 2. Observed vs. predicted feeding locations of adult sea bass derived from the random

597 forest classification model for training and testing (cross-validation) data sets.

		Predicted							
		North		Mid		South		Error (%)	
		train	test	train	test	train	test	train	test
Observed	North	28	8	3	4	8	0	28.2	33
	Mid	8	1	18	4	2	1	35.7	33
	South	7	3	4	0	38	19	22.4	13.6

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600 Table 3. Conditional variable importance from random forest analysis, expressed as the mean

601 decrease accuracy (MDA) of the random forest model associated with the removal of each

602 predictor (δ^{13}C and δ^{15}N) for each location.

	Mean decrease accuracy			
	North	Mid	South	average
δ^{15}N	33.20	5.98	21.53	31.36
δ^{13}C	14.66	26.44	37.04	47.70

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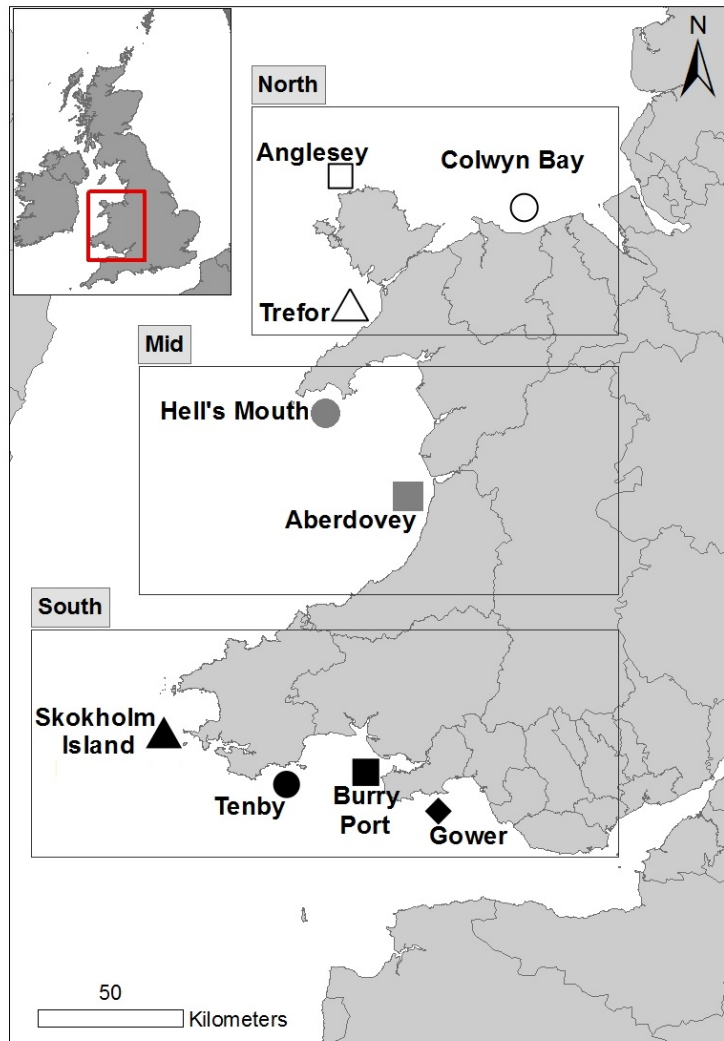
Figure 1. Map of Wales indicating the locations where sea bass were caught for the analysis of scale carbon and nitrogen stable isotope ratios.

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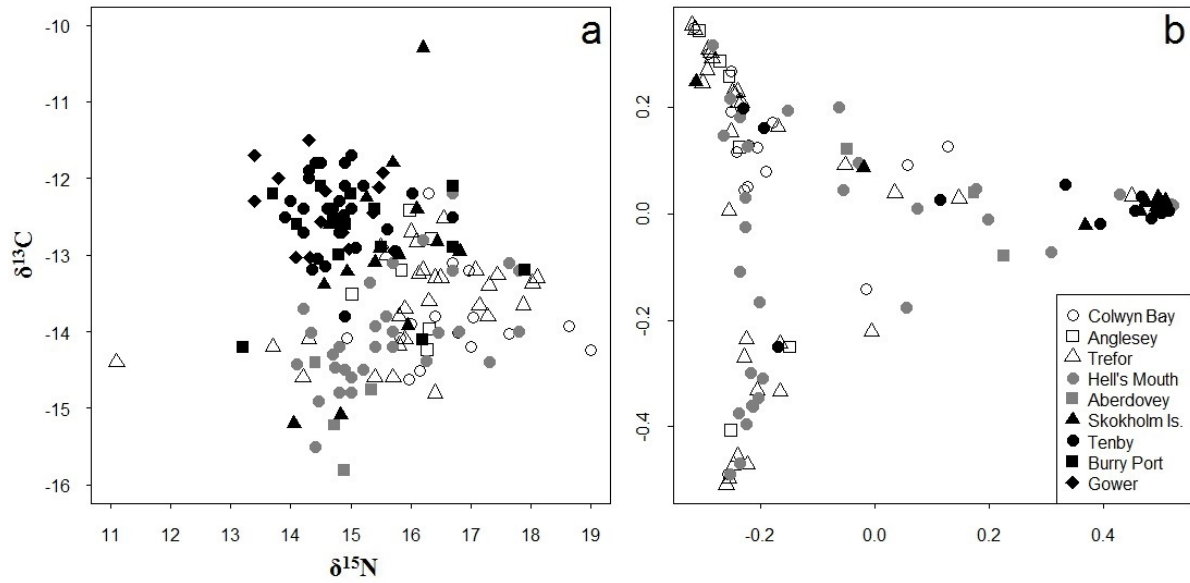
Figure 2. a) Isotopic plot showing the $\delta^{13}C$ and $\delta^{15}N$ in the scales of adult sea bass caught in nine different locations around the coast of Wales. b) Two-dimensional representation of the random forest analysis, showing the degree of separation between locations based on scale $\delta^{15}N$ and $\delta^{13}C$. Open symbols, north Wales; grey symbols, mid Wales and black symbols, south Wales.
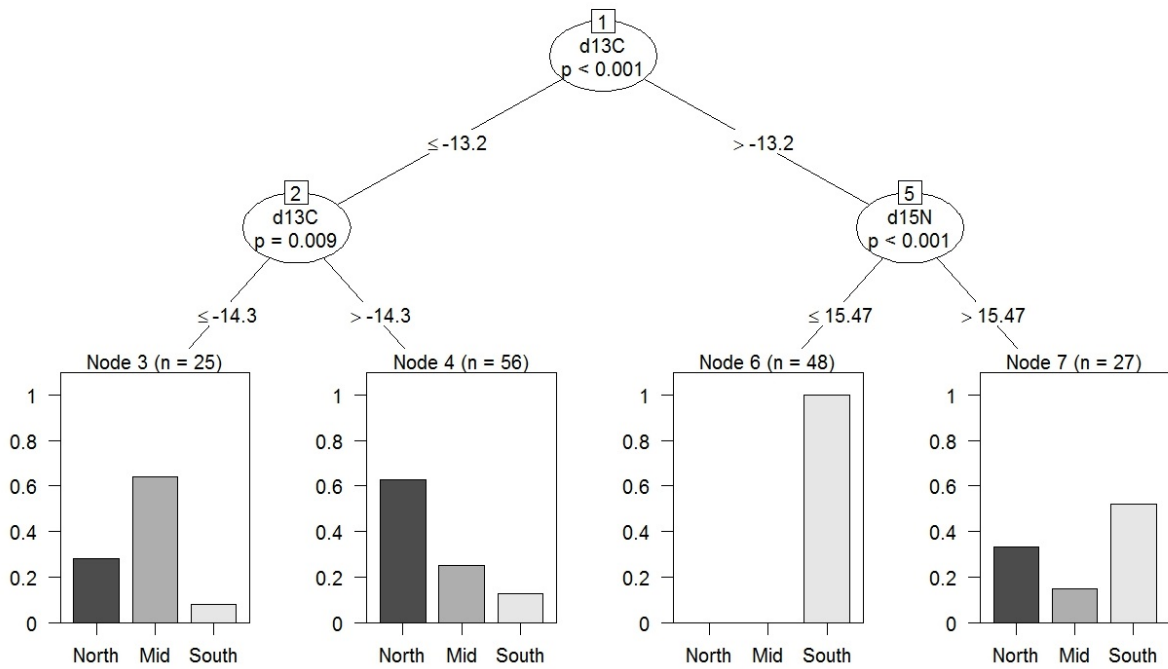
Figure 3. Conditional inference tree on the full dataset (n=156 adult bass, comprising the 116 training cases and the 40 testing cases used in the random forest analysis) using the variable $\delta^{13}C$ and $\delta^{15}N$. Inner nodes (ovals) indicate which variables were used for splitting (threshold values on the line) and n is the number of adult sea bass falling in each terminal node. Bars express the proportion of sea bass in the node that are from north, mid or south Wales.
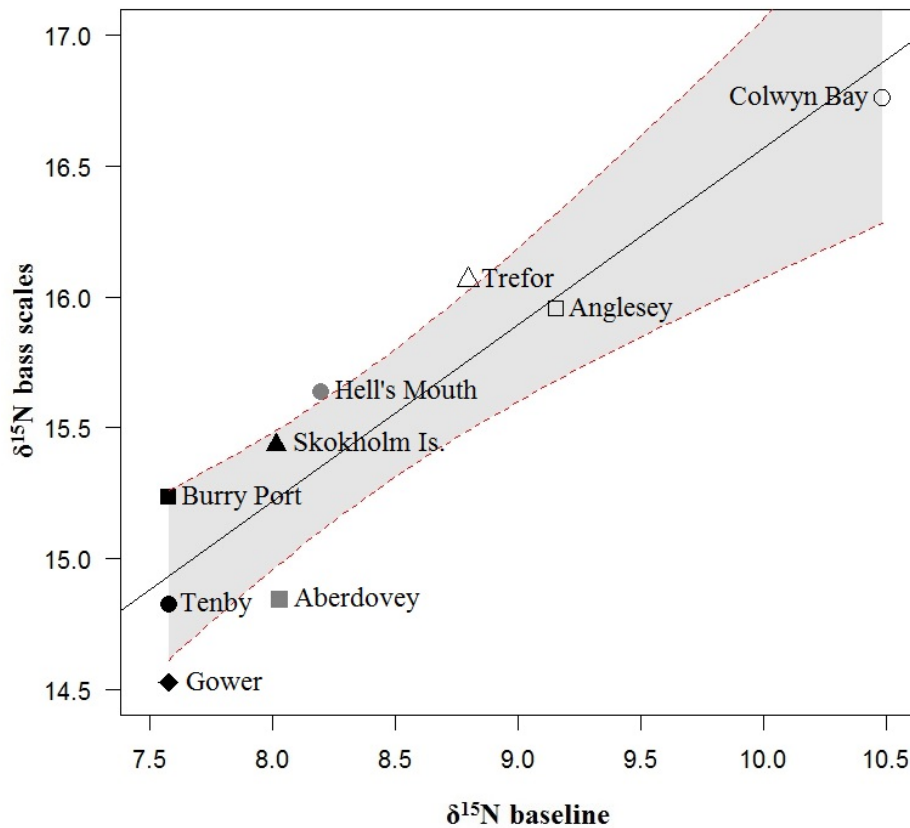
Figure 4. Relationship (regression line) between the average scale $\delta^{15}N$ in sea bass captured in 9 locations around the coast of Wales and the corresponding values of predicted spatial variation in $\delta^{15}N$ baseline estimated from the isoscape map in the Irish Sea (Jennings and Warr, 2003) (grey area: 95% confidence interval of the slope of the regression line).

640 Supplementary material

641 Supplement 1

642 Table S1. Absolute isotope values used in the repeated-measures ANOVA to assess the
643 presence of significant differences in the isotopic signature between samples of the same fish.

Location	Individual	$\delta^{15}N$	$\delta^{13}C$
Colwyn Bay	I1	17.9	-13.8
Colwyn Bay	I1	17.6	-14.1
Colwyn Bay	I1	17.5	-14.2
Trefor	I2	15.6	-13.2
Trefor	I2	16.1	-13.2
Trefor	I2	16.7	-13.3
Hell's Mouth	I3	15.7	-14.1
Hell's Mouth	I3	15.1	-13.8
Hell's Mouth	I3	15.4	-13.9
Aberdovey	I4	14.7	-15.2
Aberdovey	I4	14.8	-15.1
Aberdovey	I4	14.7	-15.0
Skokholm Island	I5	16.4	-12.8
Skokholm Island	I5	16.5	-12.8
Skokholm Island	I5	16.6	-12.9
Tenby	I6	14.8	-12.6
Tenby	I6	14.8	-12.1
Tenby	I6	15.0	-12.8
Gower	I7	15.5	-12.1
Gower	I7	15.5	-12.3
Gower	I7	15.3	-12.3

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649　Table S2. Absolute isotope values used in the one-way ANOVA to assess possible
650　differences between the feeding and spawning season for the fish caught in Hell's Mouth
651　(North Wales)

Season	Month	$\delta^{15}N$	$\delta^{13}C$
feeding	October	16.8	-14.0
feeding	October	17.8	-14.0
feeding	October	15.0	-14.6
feeding	October	14.4	-15.5
feeding	October	15.0	-14.8
feeding	November	16.5	-14.0
feeding	November	16.3	-14.4
feeding	November	15.4	-13.9
feeding	November	14.7	-14.5
feeding	November	17.6	-13.1
feeding	November	14.1	-14.4
feeding	November	15.3	-13.4
feeding	November	14.3	-14.0
feeding	November	14.5	-14.9
feeding	November	15.6	-13.8
spawning	April	15.7	-13.1
spawning	April	15.2	-14.5
spawning	April	14.2	-13.7
spawning	March	15.4	-14.2
spawning	March	14.7	-14.3
spawning	March	16.2	-12.8
spawning	March	15.7	-14.2
spawning	March	17.8	-13.2
spawning	March	14.8	-14.8
spawning	March	15.7	-14.0
spawning	March	16.7	-13.2
spawning	March	16.7	-12.2
spawning	March	14.9	-14.5
spawning	March	14.8	-14.2
spawning	March	17.3	-14.4

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654 Table S3. Absolute isotope values of the 189 sea bass collected around Wales and indication
655 of the capture location (coast vs. estuary) used for the Welch's t-test.

Area	Location	$\delta^{15}N$	$\delta^{13}C$
North	coast	14.9	-14.1
North	coast	16.8	-14.0
North	coast	19.0	-14.2
North	coast	17.0	-13.8
North	coast	16.0	-14.6
North	coast	16.1	-14.5
North	coast	17.0	-13.2
North	coast	15.8	-14.1
North	coast	17.6	-14.0
North	coast	18.6	-13.9
North	coast	16.0	-13.9
North	coast	16.3	-12.2
North	coast	17.0	-14.2
North	coast	16.4	-13.8
North	coast	16.7	-13.1
North	coast	14.7	-15.7
North	coast	16.0	-12.4
North	coast	15.8	-13.2
North	coast	16.3	-12.8
North	coast	15.0	-13.5
North	coast	16.3	-14.2
North	coast	16.3	-14.0
North	coast	16.1	-13.3
North	coast	15.9	-14.1
North	coast	16.1	-12.8
North	coast	17.1	-13.2
North	coast	17.3	-13.8

North	coast	17.9	-13.7
North	coast	15.8	-14.2
North	coast	17.4	-13.3
North	coast	16.0	-12.7
North	coast	17.3	-13.4
North	coast	15.5	-12.9
North	coast	18.1	-13.3
North	coast	16.6	-12.5
North	coast	17.1	-13.7
North	coast	18.0	-13.4
North	coast	14.3	-14.1
North	coast	16.2	-13.2
North	coast	16.3	-13.6
North	coast	16.2	-13.2
North	coast	15.7	-14.6
North	coast	15.4	-14.6
North	coast	11.1	-14.4
North	coast	14.2	-14.6
North	coast	15.9	-13.7
North	coast	13.7	-14.2
North	coast	16.4	-13.3
North	coast	16.4	-14.8
North	coast	15.6	-13.0
North	coast	16.5	-13.3
North	coast	15.8	-13.8
Mid	coast	16.5	-14.0
Mid	coast	16.3	-14.4
Mid	coast	15.4	-13.9
Mid	coast	14.7	-14.5
Mid	coast	17.6	-13.1

Mid	coast	14.1	-14.4
Mid	coast	15.3	-13.4
Mid	coast	14.3	-14.0
Mid	coast	14.5	-14.9
Mid	coast	15.6	-13.8
Mid	coast	16.8	-14.0
Mid	coast	17.8	-14.0
Mid	coast	15.0	-14.6
Mid	coast	14.4	-15.5
Mid	coast	15.0	-14.8
Mid	coast	14.7	-15.2
Mid	coast	14.4	-14.4
Mid	coast	14.9	-15.8
Mid	coast	15.3	-14.8
Mid	coast	15.2	-14.3
Mid	coast	13.9	-15.3
Mid	coast	15.4	-14.2
Mid	coast	14.7	-14.3
Mid	coast	16.2	-12.8
Mid	coast	15.7	-14.2
Mid	coast	17.8	-13.2
Mid	coast	14.8	-14.8
Mid	coast	15.7	-14.0
Mid	coast	16.7	-13.2
Mid	coast	16.7	-12.2
Mid	coast	14.9	-14.5
Mid	coast	14.8	-14.2
Mid	coast	17.3	-14.4
Mid	coast	15.7	-13.1
Mid	coast	15.2	-14.5

Mid	coast	14.2	-13.7
Mid	estuary	15.9	-19.1
Mid	estuary	15.2	-20.5
Mid	estuary	16.2	-19.5
Mid	estuary	15.5	-19.1
Mid	estuary	15.3	-19.5
Mid	estuary	15.5	-19.2
Mid	estuary	16.1	-19.1
Mid	estuary	15.3	-18.5
Mid	estuary	16.1	-19.4
Mid	estuary	15.9	-19.4
Mid	estuary	15.5	-20.1
Mid	estuary	15.6	-19.5
Mid	estuary	15.8	-19.8
South	coast	16.0	-13.9
South	coast	15.3	-12.3
South	coast	14.8	-15.1
South	coast	14.1	-15.2
South	coast	14.7	-12.6
South	coast	14.8	-12.6
South	coast	14.6	-13.4
South	coast	16.8	-13.0
South	coast	14.9	-13.2
South	coast	16.4	-12.8
South	coast	16.1	-12.4
South	coast	16.2	-10.3
South	coast	15.8	-13.0
South	coast	15.4	-13.1
South	coast	15.7	-11.8
South	coast	14.6	-13.2

South	coast	14.4	-13.1
South	coast	15.1	-12.9
South	coast	15.7	-13.0
South	coast	14.4	-13.2
South	coast	15.6	-12.7
South	coast	16.0	-12.2
South	coast	14.9	-12.5
South	coast	14.8	-12.6
South	coast	14.6	-12.4
South	coast	14.0	-12.3
South	coast	14.8	-12.3
South	coast	14.9	-13.8
South	coast	15.2	-12.1
South	coast	15.0	-12.9
South	coast	14.3	-13.0
South	coast	14.1	-13.0
South	coast	14.5	-12.6
South	coast	14.7	-12.5
South	coast	15.4	-12.5
South	coast	14.9	-12.7
South	coast	14.6	-12.2
South	coast	15.5	-11.9
South	coast	15.5	-12.1
South	coast	13.8	-12.0
South	coast	13.4	-12.3
South	coast	14.3	-11.5
South	coast	13.4	-11.7
South	estuary	15.6	-17.5
South	estuary	14.9	-16.6
South	estuary	16.7	-16.1

South	estuary	16.1	-16.2
South	estuary	14.9	-17.2
South	estuary	14.3	-20.4
South	estuary	16.5	-16.4
South	estuary	14.0	-17.6
South	estuary	14.9	-16.5
South	estuary	16.2	-16.4
South	estuary	16.1	-17.1
South	estuary	16.7	-17.9
South	estuary	15.2	-16.6
South	estuary	17.0	-19.1
South	estuary	16.7	-15.9
South	coast	14.5	-12.1
South	coast	15.5	-12.9
South	coast	15.4	-12.4
South	coast	13.7	-12.2
South	coast	16.7	-12.9
South	coast	16.2	-14.1
South	coast	17.9	-13.2
South	coast	13.2	-14.2
South	coast	14.8	-13.0
South	coast	16.7	-12.1
South	coast	15.0	-12.2
South	coast	14.7	-12.5
South	coast	14.9	-12.6
South	coast	14.1	-12.6
South	coast	15.4	-11.7
South	coast	14.2	-12.4
South	coast	14.8	-12.7
South	coast	14.9	-11.8

South	coast	14.2	-12.7
South	coast	14.8	-12.7
South	coast	14.5	-11.8
South	coast	14.3	-12.0
South	coast	14.7	-12.4
South	coast	14.3	-11.9
South	coast	14.4	-11.8
South	coast	14.9	-12.1
South	coast	15.0	-11.7
South	coast	15.0	-12.4
South	coast	13.9	-12.5
South	coast	16.7	-12.5

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