

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

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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 ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$)
22 composition of their scales. Analysis of fish scale $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ 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 $\delta^{13}\text{C}$ 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 $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ 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, Fritsch 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) (Rooper 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 $\delta^{13}\text{C}$ and otolith $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ 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 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$ of the base of the foodweb for the Irish Sea has been
127 established with a 3‰ difference in $\delta^{15}\text{N}$ 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

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

132

133 **MATERIALS AND METHODS**

134 **Data collection and preparation of samples**

135

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

140 Due to the scale architecture, using collagen from the most recent growth period is the only
141 way to obtain isotope data from the last (most recent) feeding season. Although sea bass
142 scales are relatively large, the last season is often thin (depending on the month of capture,
143 with an average width of section of 0.44 (± 1.7 SD) mm), and therefore several scales from
144 each individual fish were used to gather enough material (0.6 mg) for analysis. Individual
145 scales were briefly soaked in Millipore™ ultra-pure water and manually cleansed using non-
146 metallic forceps and a small nylon brush to remove any remaining adhering vestigial tissue.
147 The last season of growth was trimmed from the top edge of the scale, weighed and placed
148 into pre-weighed tin capsules. Decalcification of sea bass scales was not performed prior to
149 isotopic analysis since the removal of inorganic carbonates has no significant effect on scale
150 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (Sinnatamby et al. 2007, Woodcock & Walther 2014).

151 Scale samples were analysed in a mass spectrometer. Analytical precision for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$
152 was based on both the long term reproducibility of calibrated in-house standards and repeat
153 analysis of sample material. Standard deviation error for $\delta^{13}\text{C}$ was $<0.1\%$ and for $\delta^{15}\text{N}$ was
154 $<0.2\%$.

155 **Data analysis**

156

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

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

161 where X is $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ and R is the ratio of the heavy isotope to the light isotope ($^{13}\text{C}:^{12}\text{C}$ or
162 $^{15}\text{N}:^{14}\text{N}$) 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 $\delta^{13}\text{C}$ 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 $\delta^{15}\text{N}$, 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 $\delta^{13}\text{C}$ (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 $\delta^{15}\text{N}$ between individuals (e.g. Jennings et al. 2002). The
203 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ 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 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ 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 $\delta^{13}\text{C}$
216 and $\delta^{15}\text{N}$), 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 ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) 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 ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) 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 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ on
230 the classification of the feeding locations of bass and ultimately to identify the range of $\delta^{13}\text{C}$
231 and $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$ for sea bass
234 scales for each location sampled in this study and the corresponding values of predicted
235 spatial variation in $\delta^{15}\text{N}$ baseline estimated from the isoscape map (Jennings & Warr 2003).
236 The model developed by Jennings & Warr (2003), which related the $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$ ($F_{2,12}=0.33$, $p=0.73$) and $\delta^{13}\text{C}$ ($F_{2,12}=1.14$, $p=0.35$)
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 $\delta^{15}\text{N}$; $F_{1,28}=2.59$, $p=0.12$ for $\delta^{13}\text{C}$) (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 $\delta^{13}\text{C}$ values ($-18.22 \pm 1.49\text{‰}$)
260 than those caught in coastal areas ($-13.31 \pm 0.99\text{‰}$) (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 $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ 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 ($\delta^{13}\text{C}$ and
277 $\delta^{15}\text{N}$) were important for the classification process. On average, $\delta^{13}\text{C}$ seemed slightly more
278 important than $\delta^{15}\text{N}$ for classifying the fish. The importance of each isotope varied regionally
279 such that $\delta^{15}\text{N}$ was more important for classifying fish in north Wales, $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ value; sea
282 bass from north and mid Wales were allocated on the branch corresponding to $\delta^{13}\text{C} \leq -13.2\text{‰}$
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 $\delta^{13}\text{C}$ value in the range of
285 -14.3‰ and -13.2‰ while fish from mid Wales equally split between the $\delta^{13}\text{C}$ value
286 comprised between -14.3‰ and -13.2‰ (47%, $n=14$) and $\delta^{13}\text{C} \leq -14.3\text{‰}$. Only 13% ($n=9$)
287 of sea bass caught in south Wales was allocated on the branch corresponding to $\delta^{13}\text{C} \leq -$

288 13.2‰. In fact most of the sea bass from south Wales (87%) were characterized by $\delta^{13}\text{C} > -$
289 13.2‰. In particular 100% of fish with $\delta^{13}\text{C} > -13.2\text{‰}$ and $\delta^{15}\text{N} \leq 15.47\text{‰}$ 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 $\delta^{15}\text{N}$ measured in
293 this study by location (y) and the corresponding values of the $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$ detected in this
296 study are consistent with the spatial patterns in $\delta^{15}\text{N}$ 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 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of tissue protein are controlled by the trophic level of the
304 fish and $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$ found in fish scales and the
307 average value of $\delta^{15}\text{N}$ baseline by location (Jennings & Warr 2003) confirmed our
308 assumption that the observed spatial differences in sea bass $\delta^{15}\text{N}$ reflected spatial differences
309 in $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$ of sea bass along the Welsh
314 coast could be attributed to differences in base $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$ was mostly attributed to differences in $\delta^{15}\text{N}$
318 at the base of the food chain (Jennings & Warr 2003).

319 Our results also showed that the average $\delta^{15}\text{N}$ in fish scales tended to increase with latitude.
320 This south-north gradient of $\delta^{15}\text{N}$ seems to be confirmed from other studies, which
321 documented higher values of $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$ -depleted than marine plankton (Schoeninger &
330 DeNiro 1984, Owens 1987). This interpretation would also be consistent with lower $\delta^{13}\text{C}$
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 $\delta^{15}\text{N}$ appeared more important than $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ 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 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in adult sea bass collected around
351 Welsh waters showed a high degree of overlap in the isotope composition between north and

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

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

376 No information on the presence of local separate spawning areas is currently available. Due
377 to this lack of information, three possible scenarios remain open: 1) the two sub-populations
378 mix during the spawning season and are part of the same stock, 2) they do not mix during the
379 spawning season and can be therefore considered as two separate sub-populations, 3) they
380 mix only partially. Future research should address this topic and the isotopic signatures
381 identified in this study could be used to track the origin of individuals from spawning
382 aggregations. However, whichever scenario is correct, it is already clear that different spatial
383 scales of movement characterize the two sub-populations. Adult sea bass from south Wales
384 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 “Trevoise 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 Trevoise 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 $\delta^{13}\text{C}$, indicating the primary use of estuaries as feeding areas, as freshwater ecosystems
404 are generally $\delta^{13}\text{C}$ 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.

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

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

428

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437

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588 Woodcock SH, Walther BD (2014) Trace elements and stable isotopes in Atlantic tarpon
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590

591 Table 1. Values (mean \pm SD) of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ 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	$\delta^{13}\text{C}$ (VPDB) ‰	$\delta^{15}\text{N}$ (AIR) ‰
North (n=51)	Wales	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 (n=71)	Wales	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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599

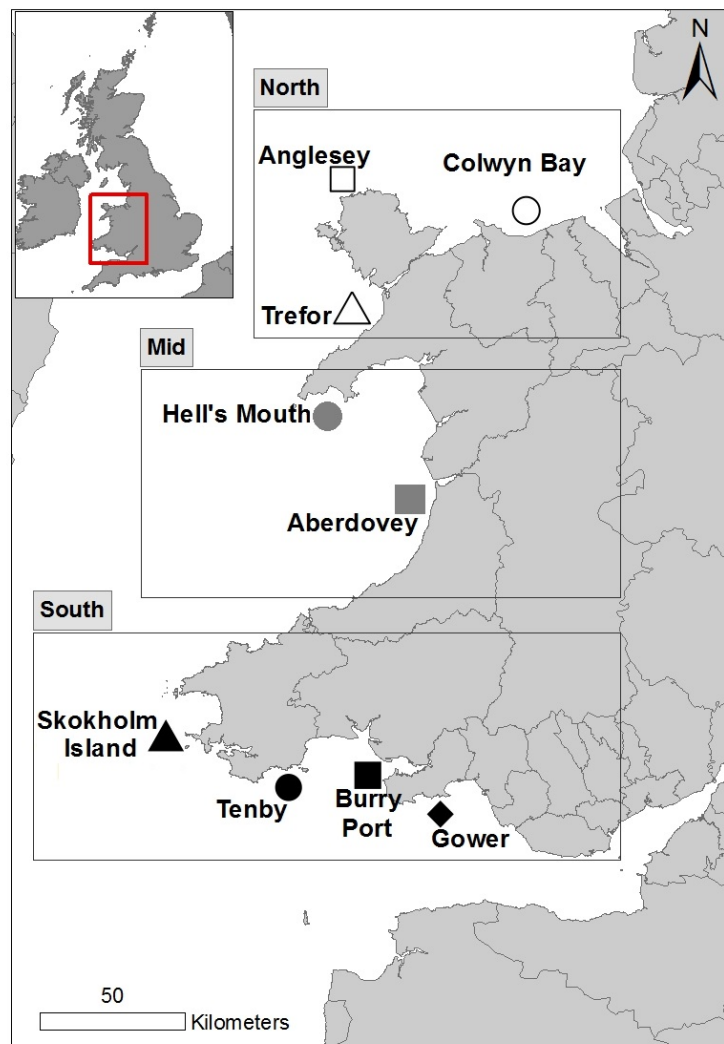
600 Table 3. Conditional variable importance from random forest analysis, expressed as the mean
601 decrease accuracy (MDA) of the random forest model associated with the removal of each
602 predictor ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) for each location.

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

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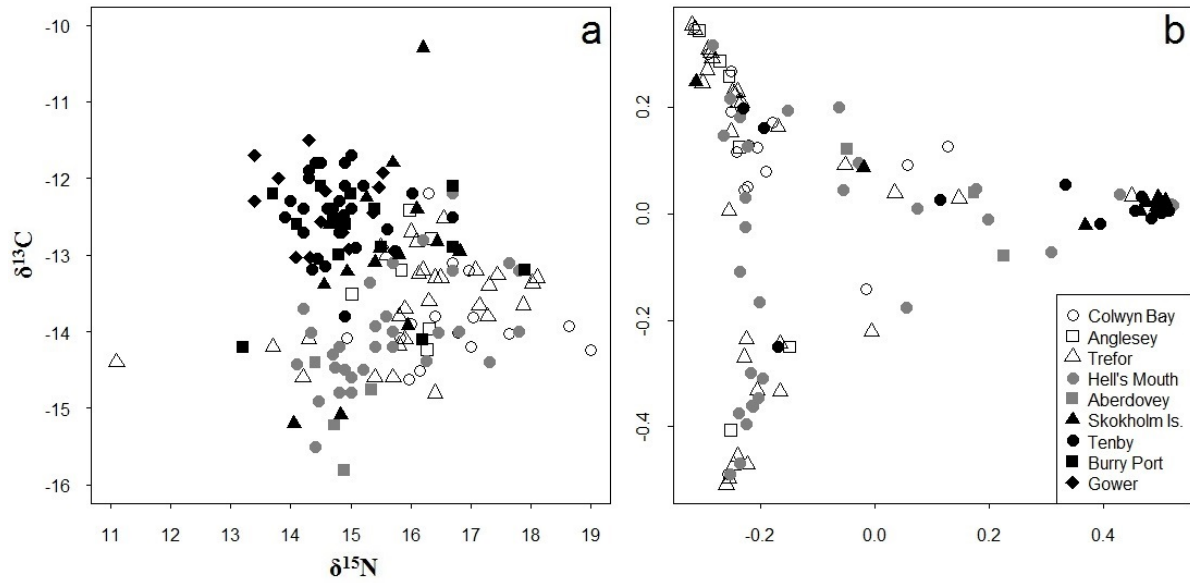
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607 Figure 1. Map of Wales indicating the locations where sea bass were caught for the analysis
608 of scale carbon and nitrogen stable isotope ratios.

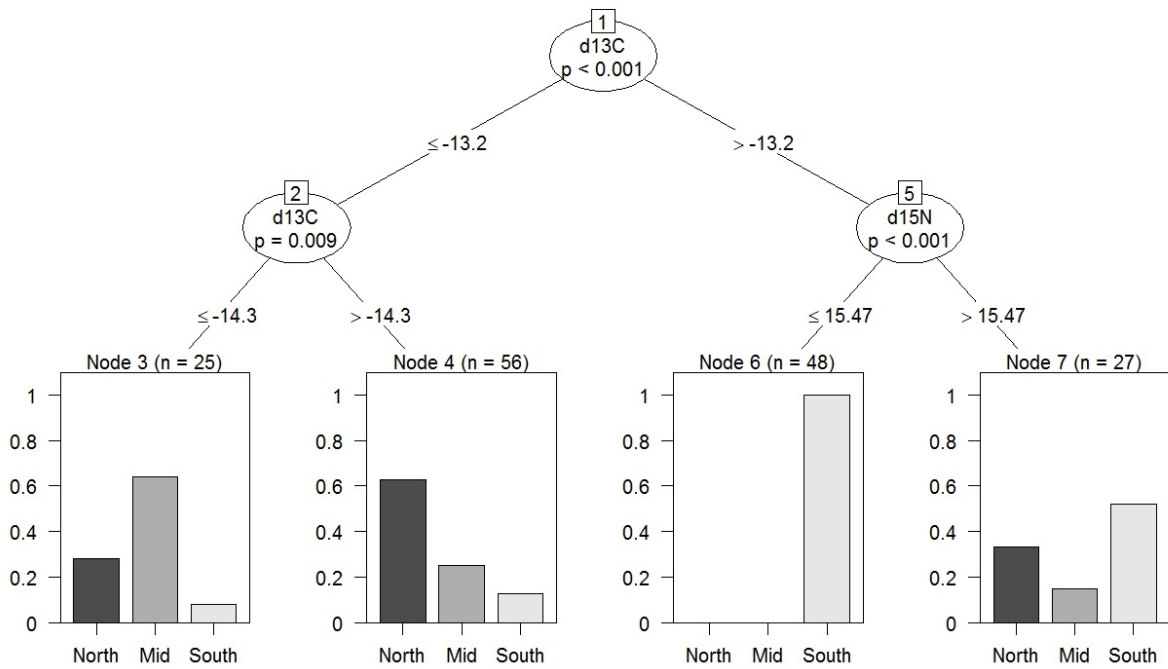
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611 Figure 2. a) Isotopic plot showing the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in the scales of adult sea bass caught in
 612 nine different locations around the coast of Wales. b) Two-dimensional representation of the
 613 random forest analysis, showing the degree of separation between locations based on scale
 614 $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$. Open symbols, north Wales; grey symbols, mid Wales and black symbols,
 615 south Wales.

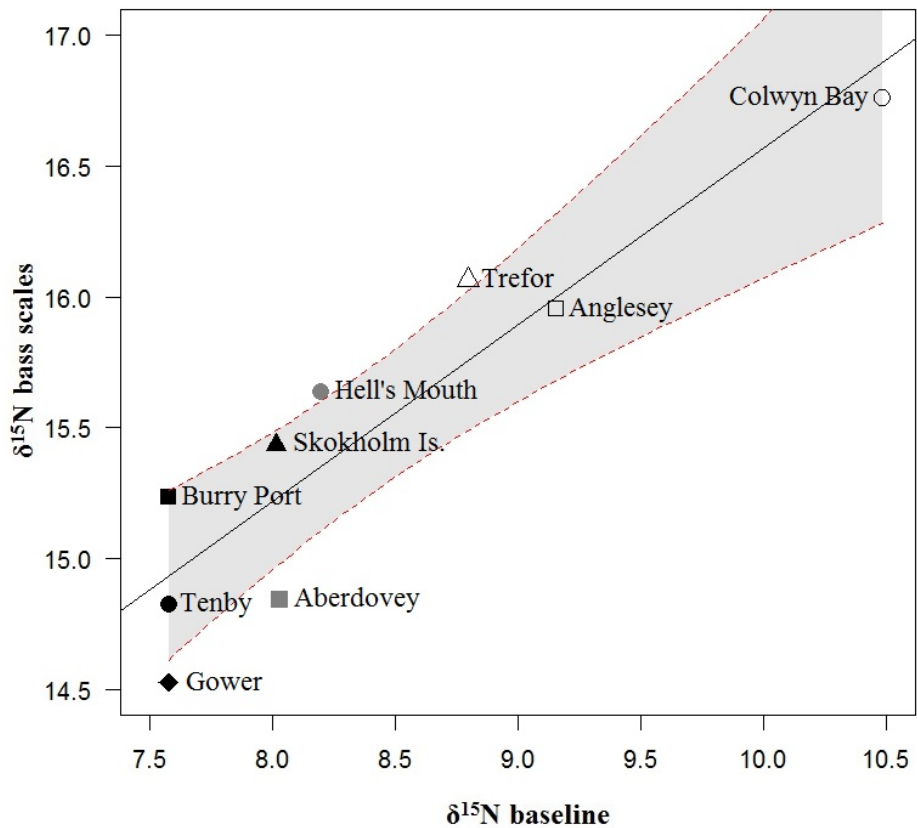
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618 Figure 3. Conditional inference tree on the full dataset (n=156 adult bass, comprising the 116
 619 training cases and the 40 testing cases used in the random forest analysis) using the variable
 620 $\delta^{13}C$ and $\delta^{15}N$. Inner nodes (ovals) indicate which variables were used for splitting (threshold
 621 values on the line) and n is the number of adult sea bass falling in each terminal node. Bars
 622 express the proportion of sea bass in the node that are from north, mid or south Wales.

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625 Figure 4. Relationship (regression line) between the average scale $\delta^{15}\text{N}$ in sea bass captured
 626 in 9 locations around the coast of Wales and the corresponding values of predicted spatial
 627 variation in $\delta^{15}\text{N}$ baseline estimated from the isoscape map in the Irish Sea (Jennings and
 628 Warr, 2003) (grey area: 95% confidence interval of the slope of the regression line).

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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	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$
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	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$
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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653

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	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$
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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