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

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Running head: Isotopic signature in sea bass populations

ABSTRACT

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

waters use separate feeding grounds (south vs. mid/north Wales), and may need separate management.

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

INTRODUCTION

Most current fisheries controls are applied at large geographic scales that often encompass entire sea basins. Effort controls such as Minimum Landing Size (MLS) are only effective if the life history and growth of fish is uniform across the scale at which they are applied. However, for many species of fish, we have limited understanding of their home range and hence the interaction between local environmental parameters and life history traits such as growth rate potentially leading to a mismatch with the scale of management.

The European sea bass (*Dicentrarchus labrax*) is an economically important species exploited by multiple fishing fleets across Europe (Pawson et al. 2007a, ICES 2013). In northern Europe, the recent combination of declining recruitment and increasing fishing mortality has led to a rapid decline in stock biomass and has triggered management advice for an 80% reduction in catches as an immediate conservation measure (ICES 2014). A package of emergency management measures was implemented in 2015 and then strengthened for 2016. For commercial fishing, these measures included a temporal ban on pelagic trawling (six months) and on hooks, lines and fixed gill nets (two months), a monthly catch limit, an increase in the MLS from 36 to 42 cm for northern sea bass and an area closure around Ireland. For recreational fishers the measures include a six month moratorium followed by one fish bag limit and the same increase of the MLS of the commercial sector (http://ec.europa.eu/fisheries/cfp/fishing_rules/sea-bass/index_en.htm).

Although the stock structure of sea bass in the northeast Atlantic has not been clearly delineated, there is evidence that sea bass around Ireland and in the Bay of Biscay could be treated as two populations separate from the eastern Celtic Sea, English Channel, and North Sea (Fritsch et al. 2007, ICES 2012). In addition, previous proposals of stock boundaries, based on conventional tagging studies (ICES 2001, ICES 2002, ICES 2004, Fritsch et al. 2007, Pawson et al. 2007b), concluded that there were two separate stock units between the east and west UK (Pawson et al. 2007b). However, despite the inferences from tagging studies, ICES has

concluded that current evidence supports the view that sea bass in the North Sea (ICES Division IVb&c) and in the Irish Sea, the English Channel and Celtic Sea (Ices Divisions VIIa,d,e,f,g&h) should be treated as a functional stock unit as there is no clear basis at present to subdivide them into independent stock units (ICES 2012). Clearly, a more accurate definition of the stock boundaries is required to improve assessment and management advice to underpin sustainable exploitation.

Previous tagging studies around England and Wales have shown a tendency for adult sea bass to migrate to the south and west in autumn (English Channel), during the pre-spawning season, and to return north and eastwards in spring to feeding areas (Pawson et al. 2007b, Pawson et al. 2008). These mark-recapture studies have provided evidence of philopatry in relation to feeding and spawning areas (Pawson et al. 2007b, Pawson et al. 2008). Pawson et al. (2008) reported that 55% of sea bass > 40 cm that were tagged and released during the summer were subsequently recaptured within 16 km of their original tagging location on their summer feeding grounds. A further 23% were recaptured during winter at least 80 km from their release site. These data provided strong evidence that sea bass in England and Wales may share common migration routes to the same spawning grounds but may exhibit segregation by returning to specific summer feeding grounds (Pawson et al. 2008). If this is the case, then management of sea bass stocks may need to be applied at a much smaller regional level than currently proposed by ICES.

Although the use of external tags can provide valuable information on stock structure and movement patterns of fish (e.g. Dunn and Pawson 2002, Pawson et al. 2007b, Neuenfeldt et al. 2013) there are multiple problems with their use that include, for example, transmission of data to the researchers, and poor recapture rates (Block et al. 2011). Natural biogeochemical markers (trace elements and stable isotopes) located in the hard parts of fishes (e.g otoliths and scales) have great potential as ‘internal’ tags to study stock structure, and to study connectivity between fish populations in, and movements between, chemically distinctive water bodies by fishes during their lifetime (Elsdon et al. 2008, Trueman et al. 2012). Structures such as otoliths and scales deposit new material incrementally as the fish grows, and can provide a record of the elemental and isotopic composition of the water in which the fish has lived at each stage of its lifetime (e.g. Thorrold et al. 1998, Cadrin et al. 2013).

The use of stable isotopes remains a relatively underused tool for tracking migration and general movements in marine animals (Trueman et al. 2012). This is because there can be

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

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

The aims of this paper were to (1) measure the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ composition in the last season of growth in scales from adult sea bass caught at several locations around Wales (UK) and (2) to use these isotopic signatures to infer patterns of movement and connectivity between feeding areas to determine the possible presence of different stock units within Welsh coastal waters. Spatial variation in $\delta^{15}\text{N}$ of the base of the foodweb for the Irish Sea has been established with a 3‰ difference in $\delta^{15}\text{N}$ between north and south Wales (Jennings & Warr 2003). Therefore, if sea bass show philopatry to regional feeding grounds around the UK, as 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 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (Sinnatamby et al. 2007, Woodcock & Walther 2014).

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

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:

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

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

We first measured the intra fish variability to determine whether the use of a single sample per fish provided an accurate measurement of the isotopic signal. We tested seven fish (three from north, one from mid and three from south Wales). For each fish, we prepared and compared three replicates of scale material (last growing season) of 0.6 mg obtained from the same single mix of scales collected from the body area under the pectoral fin. A repeated-measures ANOVA was then applied to assess the presence of significant differences in the isotopic signal between samples of the same fish.

Of the 189 fish whose samples were analysed in the present study, 28 (38.6-60.7 cm TL) were caught in estuaries (Aberdovey-mid Wales, n=13 and Burry Port-south Wales, n=15) while the remainder were caught in open coastal waters. A comparison of the $\delta^{13}\text{C}$ values between the two groups (estuarine fish vs. marine fish) was undertaken using Welch's t-test. This test was not performed for $\delta^{15}\text{N}$, due to the influence of the fish size on the accumulation of the isotope ratio of this element (13 estuarine fish were > 50 cm TL) (Jennings et al. 2002). To assess the possible differences in the isotope signal of sea bass collected during the feeding and spawning season, sea bass from the same location (Hell's Mouth-Mid Wales, fish provided by the same fisher and caught in the same spot, n=15 for both seasons) were compared using a one-way ANOVA. This analysis explores how long sea bass stay in the same feeding area. As the scale material is laid down during the feeding season, the scales collected during the spawning season represent the isotopic signature of the area occupied by the fish during the whole preceding feeding season and not only during part of it.

To assess the presence of spatial differences in the isotope composition between areas a random forest classification model (R package "randomForest", Liaw & Weiner 2002) was used. Random forest analysis (Breiman 2001) is a nonparametric technique derived from classification and regression trees (CART). The decision-tree modelling approach requires fewer assumptions than traditional parametric methods (e.g. Linear Discriminant Analysis) (Strobl et al. 2009). In particular random forest analysis allows correlated predictor variables to be utilised without transformation or exclusion to obtain unbiased predictions and estimates of variable importance (Strobl et al. 2009). In this context, random forest analysis has been shown to be preferred for discrimination based on otolith microchemistry when the assumptions of the traditional parametric methods cannot be reached (Mercier et al. 2011). The

random forest model produces many classification trees from which are derived an ensemble of classifications to predict the dependent variable (in our case “geographic location” of sea bass) as a result of average assignment across trees (Strobl et al. 2007, Strobl et al. 2009). By default, the random forest model partitions the data into ‘training’ (generally 70% of data) and ‘test’ samples selected at random from the data set. Whilst the training samples are used to build the model, the test set is used to validate its performance.

Estuarine fish were excluded from this analysis, to remove the effect of the different salinities encountered between freshwater and marine habitats on $\delta^{13}\text{C}$ (Doucett et al. 1999). The analysis was also restricted to fish of a similar size range (40-50 cm TL) to remove the effect of the fish size on the variation of $\delta^{15}\text{N}$ between individuals (e.g. Jennings et al. 2002). The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of 156 sea bass between 40 and 50 cm TL caught in coastal areas (98 caught during the feeding season and 58 during the spawning season) were then compared to assess if spatial differences in isotope composition existed. The nine different capture locations were first aggregated into three main groups: north, mid and south Wales (Figure 1). The random forest classification model was then used to test if there was a difference in scale $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values between north, mid and south Wales and whether it was possible to correctly assign the fish to the area where it was caught based on scale isotopic signature. In addition this statistical technique allowed the importance of each predictor variable in the classification process to be evaluated and ultimately to identify specific isotopic signatures by area. Based on the level of separation and/or overlap of the isotopic signatures between areas we expected to derive insights on movement patterns related to feeding behaviour for adult sea bass.

The association, or proximity, between each fish (characterized by the combination of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$), is the number of times that they occur together in the same terminal node. The “randomForest” package normalized these counts to produce a proximity matrix that can be analysed using a metric scaling method. The resultant Multi-Dimensional Scaling plot represented the degree of differentiation in stable isotopes values between the three geographic locations.

Conditional variable importance was reported to show the relative contribution of each predictor variable ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) to the classification performance. To evaluate the conditional variable importance we measured the Mean Decrease Accuracy (MDA) of the forest when the values of each predictor ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) are randomly excluded (or permuted). The greater the

decrease in the accuracy of the random forest resulting from the exclusion (or permutation) of a single variable, the more important that variable is for classification of the data.

To aid interpretation of the results of the random forest analysis, a conditional inference tree was used. In particular this single-tree method helped to trace the effects of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ on the classification of the feeding locations of bass and ultimately to identify the range of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values associated with each location. To this end, the “ctree” function for conditional inference trees in the “party” R package (Hothorn et al. 2006) was used.

We finally assessed the degree of correlation between the average value of $\delta^{15}\text{N}$ for sea bass scales for each location sampled in this study and the corresponding values of predicted spatial variation in $\delta^{15}\text{N}$ baseline estimated from the isoscape map (Jennings & Warr 2003). The model developed by Jennings & Warr (2003), which related the $\delta^{15}\text{N}$ of scallops of each sampling site to day of sampling, shell height, depth, surface temperature, bottom temperature and summer salinity was used to calculate the $\delta^{15}\text{N}$ baseline corresponding to our capture locations. Since not all our sampling sites corresponded exactly to the scallop samplings sites in Jennings & Warr (2003), we used the $\delta^{15}\text{N}$ baseline values corresponding to the closest areas to our sampling sites (Colwyn Bay, site 28; Anglesey, site 27; Trefor, site 30; Hells Mouth, site 31; Aberdovey, site 33; Skokholm Island, site 34; Tenby, Burry Port and Gower, site 37). Although this isoscape map was developed in 2002, the hydrodynamic and biogeochemical processes controlling the distribution of carbon and nitrogen isotope values are temporally stable (MacKenzie et al. 2014). All analyses were carried out using R version 3.0.2 (R Core Team 2013).

RESULTS

The repeated measures ANOVA showed no significant difference between the three replicates of the scale material in $\delta^{15}\text{N}$ ($F_{2,12}=0.33$, $p=0.73$) and $\delta^{13}\text{C}$ ($F_{2,12}=1.14$, $p=0.35$) values (Table S1 in Supplement 1). This demonstrated that the use of a single sample per fish provided an accurate measurement of the isotopic signal in scale material laid down during the last feeding season.

No significant difference was found in the isotope data between the feeding and spawning season for the fish caught in Hell’s Mouth (North Wales) (One-way ANOVA, $F_{1,28}=0.17$, $p=0.68$ 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

Supplement 1). This result suggested that adult sea bass caught in the same area have similar isotope composition even when caught at different times of the year.

The 28 sea bass caught in estuaries had a significantly lower $\delta^{13}\text{C}$ values ($-18.22 \pm 1.49\text{‰}$) than those caught in coastal areas ($-13.31 \pm 0.99\text{‰}$) (Welch's t-test, $t = 16.8$, $P < 0.001$; absolute values are reported in Table S3 in Supplement 1) which was expected given that freshwater ecosystems generally have lower $\delta^{13}\text{C}$ values relative to marine systems (Doucett et al. 1999). Out of these fish, 13 were adult sea bass > 50 cm TL.

Regional difference in the marine $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values was detected (Table 1), such that values from south Wales were separated from those of mid and north Wales (Figure 2a). The two dimensional representation of the random forest analysis also showed the degree of overlap of the isotopic signature between north and mid Wales (Figure 2b).

The random forest classification model built on the training samples had a discrimination capacity c. 75%, which means that most fish were correctly classified between north, mid and south Wales. For both the training ($n=116$) and test ($n=40$) data sets, the majority of the misclassifications were fish from north Wales that were classified as mid Wales and vice versa, while the majority of fish from south Wales were correctly assigned (80%) (Table 2). In this case, the misclassification corresponded to regions (Tenby and Skokholm Island) geographically closer to Mid Wales than the rest of the samples (Burry Port and Oxwich-Gower).

The Mean Decrease Accuracy (MDA) of the forest showed that both variables ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) were important for the classification process. On average, $\delta^{13}\text{C}$ seemed slightly more important than $\delta^{15}\text{N}$ for classifying the fish. The importance of each isotope varied regionally such that $\delta^{15}\text{N}$ was more important for classifying fish in north Wales, $\delta^{13}\text{C}$ for mid Wales and both isotopes were important for classifying fish to south Wales (Table 3).

The most important primary split in the conditional inference tree was the $\delta^{13}\text{C}$ value; sea bass from north and mid Wales were allocated on the branch corresponding to $\delta^{13}\text{C} \leq -13.2\text{‰}$ on the proportion of 82% ($n=42$) and 88% ($n=30$) respectively (Figure 3). Out of these proportions, most of the fish from north Wales (83%, $n=35$) had a $\delta^{13}\text{C}$ value in the range of -14.3‰ and -13.2‰ while fish from mid Wales equally split between the $\delta^{13}\text{C}$ value comprised between -14.3‰ and -13.2‰ (47%, $n=14$) and $\delta^{13}\text{C} \leq -14.3\text{‰}$. Only 13% ($n=9$) of sea bass caught in south Wales was allocated on the branch corresponding to $\delta^{13}\text{C} \leq -13.2\text{‰}$. In fact most of the sea bass from south Wales (87%) were characterized by $\delta^{13}\text{C} > -13.2\text{‰}$. 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 Wales. This specific signature characterised 68% of the total samples from south Wales (Figure 3).

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

DISCUSSION

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

Our results also showed that the average $\delta^{15}\text{N}$ in fish scales tended to increase with latitude. This south-north gradient of $\delta^{15}\text{N}$ seems to be confirmed from other studies, which documented higher values of $\delta^{15}\text{N}$ associated with cooler temperatures (Jennings et al. 2008) and higher

levels of oxygen (Radabaugh et al. 2013). While the mechanisms underpinning the spatial isotopic gradients are not fully understood and several factors may influence the spatial variation in $\delta^{15}\text{N}$ values, the degree of resuspension of particulate organic nitrogen is likely to be a major factor (e.g. Saino & Hattori 1987). Aberdovey (mid Wales) was the only location where sea bass had lower $\delta^{15}\text{N}$ compared to two areas further south. It is possible that the sea bass from Aberdovey (a site at the mouth of a large estuary) were caught along a freshwater-marine gradient, a fact that could explain this apparent discrepancy, as terrestrial inputs to freshwater bodies are more $\delta^{15}\text{N}$ -depleted than marine plankton (Schoeninger & DeNiro 1984, Owens 1987). This interpretation would also be consistent with lower $\delta^{13}\text{C}$ with respect to fish from the other coastal areas, which could confirm that sea bass caught in Aberdovey originated from a feeding area with freshwater influence (Doucett et al. 1999).

Our study also demonstrates that the random forest analysis, although not yet widely used in marine ecology for hierarchical classification (e.g. Mercier et al. 2011), is a powerful statistical tool, as it allows not only predictors to be used without transformation or exclusion but also the relative importance of the variables in the classification process to be estimated (Strobl et al. 2009). Therefore, while $\delta^{15}\text{N}$ appeared more important than $\delta^{13}\text{C}$ in classifying adult sea bass in north Wales (c.f. fish from mid Wales), both variables were necessary to correctly classify fish from south Wales. Moreover the conditional inference tree allowed us to identify the range of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values associated with each location and thus to establish a geographic range of the isotopic signatures. Additional variables (e.g. element concentration, Sr, Ba, Mn and Mg) could be used in the future to improve the discriminatory power of the random forest and thus the precision level of the biogeochemical tag (Wells et al. 2000, Ramsay et al. 2011, Seeley et.al 2015).

Our results showed that the stable isotope composition of fish scales provides unique insights into fish movement at a regional scale (e.g. the Welsh coastline) without the need for expensive conventional tagging studies. While fine-scale ontogenetic changes in habitat use have been identified for other fish species (e.g. NE Atlantic orange roughly, Shephard et al. (2007)), the use of fish scales has been limited to few species to date (Ramsay et al. 2012; Woodcock & Walther 2014). The analysis of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in adult sea bass collected around 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 demonstrated by this study but also for spawning, considering the proximity of a well-known spawning ground of the species outside the Bristol Channel (known as "Trevose Head", Lancaster et al. 1998, Reynolds et al. 2003). In this sense, the south Wales sub-population would have a "resident"

behaviour, where feeding grounds and spawning grounds are all within close geographical proximity to one another. This trait has been found in many populations of the Atlantic cod *Gadus morhua* where the residence areas (nursery, feeding and spawning grounds) were geographically close and had relatively stable environmental conditions (Robichaud & Rose 2004). In contrast, adult sea bass from mid and north Wales either undertake a long-distance migration to the Trevoise Head spawning area and/or other potential spawning grounds in south Wales/England or, alternatively, a small-scale migration to possible spawning grounds located off the coast of mid and/or north Wales. Our results provide for the first time insights into a regional segregation of the sea bass stock, which implies that local/regional management may be more appropriate than the present single stock approach. In this context, specific regulations based on regional variation in the ecology and life history of sea bass could facilitate a more rapid achievement of the conservation objectives.

Our results also suggest that a portion of the largest sea bass adopt estuaries as preferential feeding areas. In fact all fish with a total length >50 cm caught in estuarine areas showed very low $\delta^{13}\text{C}$, indicating the primary use of estuaries as feeding areas, as freshwater ecosystems are generally $\delta^{13}\text{C}$ depleted relative to marine systems (Doucett et al. 1999). It is possible that interspecific competition for resources has resulted in some big adult sea bass adopting an estuarine feeding habit. Estuaries have been recognised to support the early phase of development of the species (Kelley 1988, Lancaster et al. 1998) as well as part of the juvenile stock (3-5 years old) (Pawson et al. 2005) and the present study stressed the importance of this habitat for large adult sea bass. While other fish species, such as flathead mullet *Mugil cephalus*, have been recognised as using estuarine areas through the juvenile and/or adult life stages (Elliott et al. 2007), few previous studies have mentioned the presence of adult sea bass in estuarine habitats in NE Atlantic (Kelley 1987, Kennedy & Fitzmaurice 1972, Pickett & Pawson 1994). Moreover, these studies focused on describing the opportunistic feeding behaviour of the species and its different habitat use, without associating estuaries with a specific ecological role in the adult phase. Our results indicated estuarine feeding of some large sea bass during extended time periods, possibly during the whole feeding season. 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 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ 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.

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Table 1. Values (mean \pm SD) of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in scale material formed during the last growth period of the adult sea bass *Dicentrarchus labrax* (40-50 cm TL, n=156) caught in coastal areas around Wales.

Area	Location	$\delta^{13}\text{C}$ (VPDB)‰	$\delta^{15}\text{N}$ (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

588 Table 2. Observed vs. predicted feeding locations of adult sea bass derived from the random
 589 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

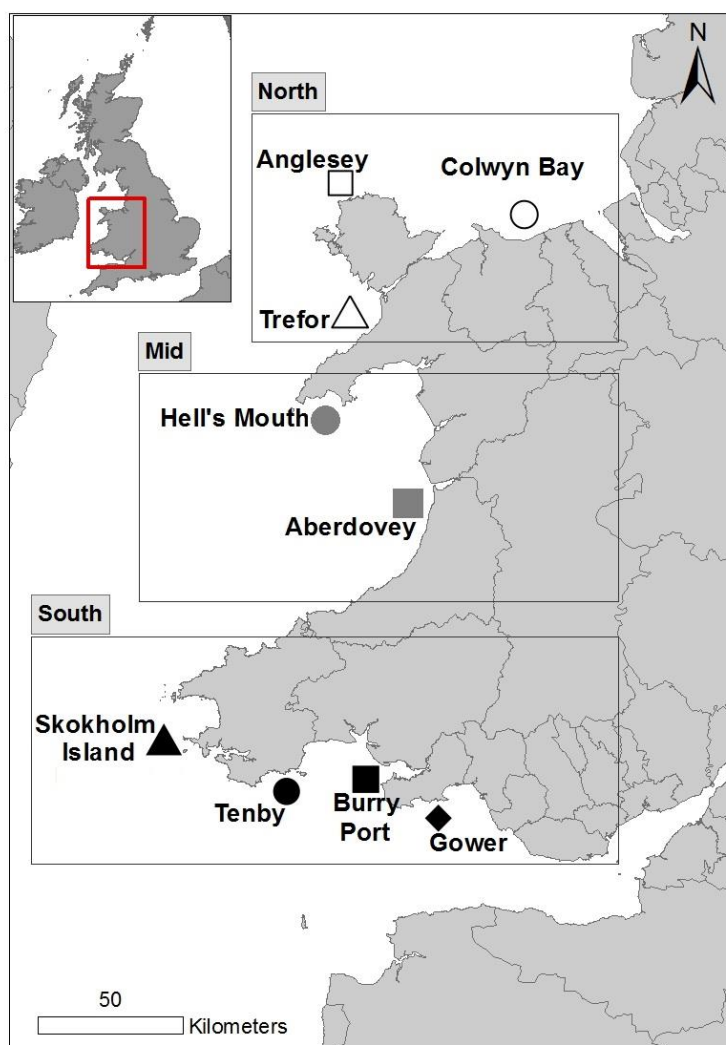
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Table 3. Conditional variable importance from random forest analysis, expressed as the mean decrease accuracy (MDA) of the random forest model associated with the removal of each predictor ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) for each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

597



598

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

601

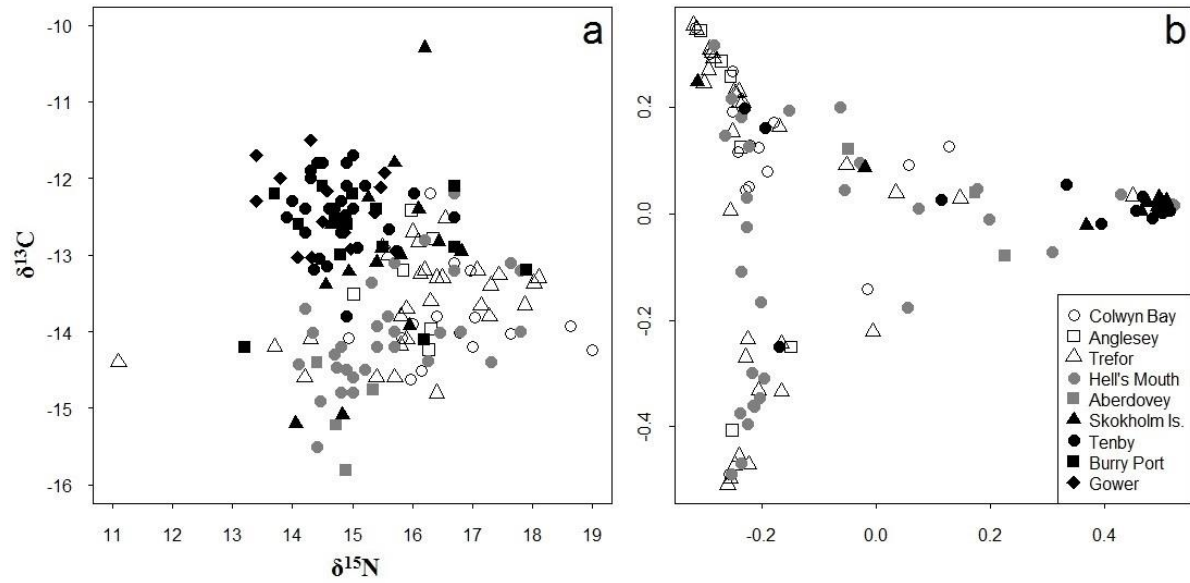


Figure 2. a) Isotopic plot showing the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in the scales of adult sea bass caught in nine different locations around the coast of Wales. b) Two-dimensional representation of the random forest analysis, showing the degree of separation between locations based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

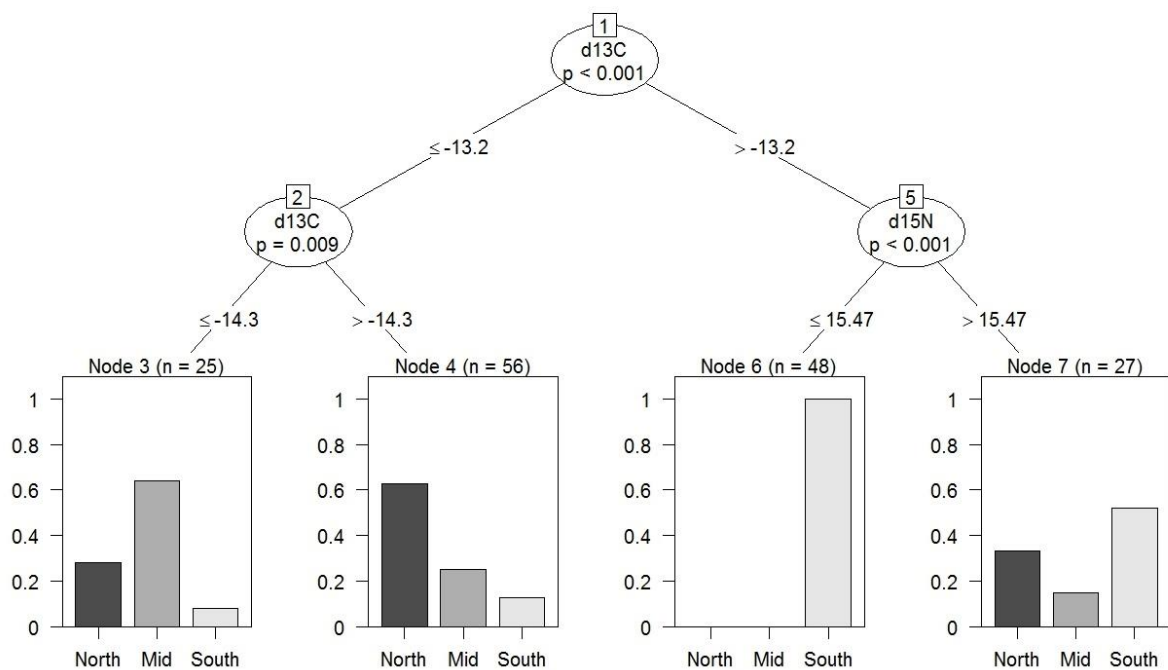


Figure 3. Conditional inference tree on the full dataset ($n=156$ adult bass, comprising the 116 training cases and the 40 testing cases used in the random forest analysis) using the variable $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. Inner nodes (ovals) indicate which variables were used for splitting (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.

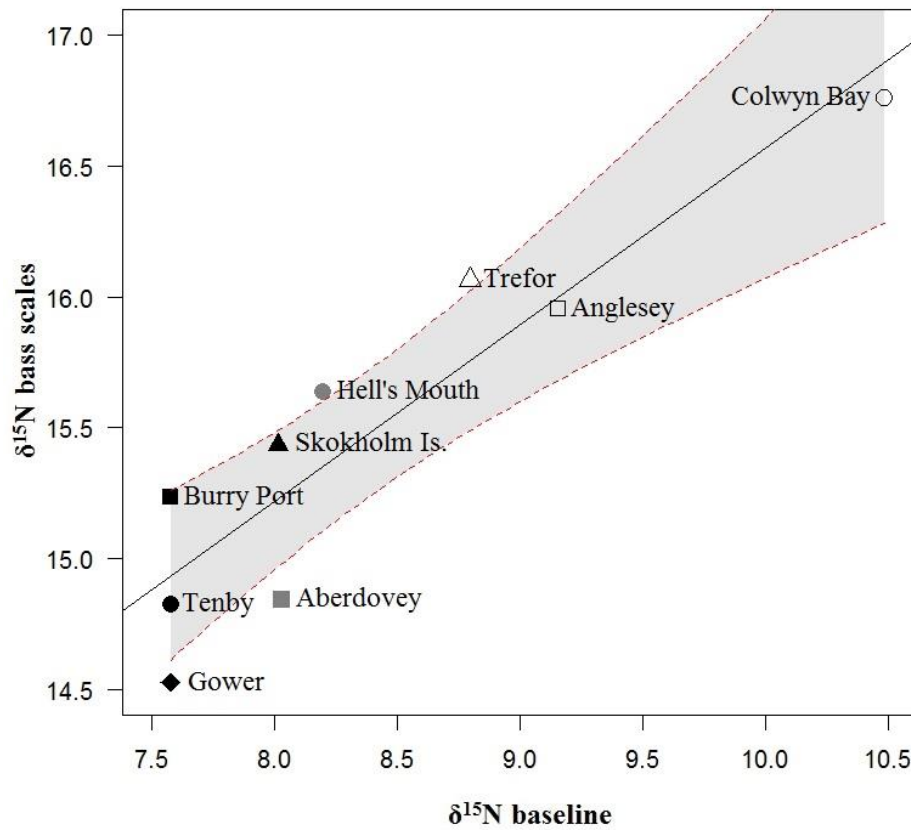


Figure 4. Relationship (regression line) between the average scale $\delta^{15}\text{N}$ in sea bass captured in 9 locations around the coast of Wales and the corresponding values of predicted spatial variation in $\delta^{15}\text{N}$ baseline estimated from the isoscape map in the Irish Sea (Jennings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

Supplementary material

Supplement 1

Table S1. Absolute isotope values used in the repeated-measures ANOVA to assess the 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

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

645 Table S3. Absolute isotope values of the 189 sea bass collected around Wales and indication
646 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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