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Debunking paradigms in estuarine fish species richness

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Running page header: Estuarine Fish Latitude Size Relationship

Abstract

The comparison of species complements within and between habitats and geographical areas is a fundamental aspect of ecological assessments. However, many influences resulting from variability in sampling and data analysis often hinder the ability to determine important patterns in community structure. The study is based on the hypothesis that using a standard sampling method, an asymptote in the rarefaction curve represents the total (gear-specific) species complement likely to be encountered for the geographical area. Accordingly, an asymptotic species richness estimator was used to predict the full complement of species present within each estuary that could be caught using seine netting. The rarefaction curves and species richness estimator enable the interrogation of two underlying paradigms of ecological species richness: the species-energy relationship and the species-area relationship. This analysis reveals distinct groups which show a significant relationship with latitude and size, although the size effect has a smaller influence. In particular, the species-latitude relationship paradigm holds true in this study while the species-area relationship paradigm only applies when latitude is considered concomitantly. Marine species in particular appear to account for the increased fish species number at lower latitudes. The underlying influence of latitude and estuary size suggests that any managerial tool that explores anthropogenic impacts (such as those used in the European Water Framework Directive) should include these aspects. It is concluded that the analysis gives environmental managers an objective cost-beneficial method of identifying when and where further sampling does not give further information for management.

Key words: seine netting; rarefaction curves; fish species richness; species-energy relationship; species-area relationship

Introduction

The importance of estuaries for freshwater, migratory, estuarine and many marine fish species is well described (Elliott et al. 2007a, Nicolas et al. 2010b) with their highly variable environments providing essential breeding, feeding and nursery habitats (Potts & Swaby 1993, Elliott et al. 2002, Elliott & Whitfield 2011, Potter et al. 2015). Estuaries and their catchments also support large urban and industrial areas, containing anthropogenic activities and pressures associated with development (McLusky & Elliott 2004, Cardoso et al. 2011, Vasconcelos et al. 2015).

Continued and recent requirements for effective management have led to the exploration of the relationships between biogeography, geomorphology and fish diversity in estuaries at global, regional and local scales (Pasquaud et al. 2015, Vasconcelos et al. 2015, França & Cabral 2015). Exploration of the complex nature of factors, including the controlling hydrophysical elements that can affect fishes in estuaries, suggest two underlying fundamental paradigms that aim to explain the fish species richness in estuaries. Firstly, species richness appears to increase with waterbody size (Gleason 1922, Nicolas et al. 2010b) in which the species-area relationship (SAR) assumes that larger waterbodies support a higher number of species as they likely provide a greater diversity of habitats and therefore a higher availability of ecological niches (Pease 1999, Perez-Ruzafa et al. 2007, Franco et al. 2008a). Secondly, species richness appears to decrease with increasing latitude (Gaston 2000, 2007, Vasconcelos et al. 2015). This relationship, henceforth identified as the species-latitude relationship (SLR), has been confirmed for estuarine fish assemblages investigated at the global scale (Pasquaud et al. 2015, Vasconcelos et al. 2015). Although not so for fishes its validity at a smaller geographical scale (spanning less than three degrees in latitude) has been shown for other groups (Gotelli & Ellison 2002). The SLR relates generally to the balance between the speciation/immigration and extinction/emigration of species resulting from the combination of multiple mechanisms, including geographic area, productivity, ambient energy and evolutionary speed among others (Willig et al. 2003).

Species richness is a metric commonly used to assess the status of estuarine fish assemblages across North East Atlantic (Perez-Dominguez et al. 2012, Lepage et al. 2016), under the requirements of the European Union Water Framework Directive (WFD, 2000/60/EC) that a ‘good ecological and chemical status’ is achieved in all European waterbodies. Where this condition is not met, management measures are to be implemented, and therefore it is of paramount importance that the assessment is based on a good understanding of the structure and functioning of the system under management and that appropriate and sound indicators are used (Hering et al. 2010). These indicators (e.g. fish species richness) need to be independent from confounding factors as for example variable sampling effort that might mask the actual variability of the metric in relation to waterbody characteristics and therefore lead to biased assessments of the ecological status (Elliott et al. 2006). Many of the WFD tools developed to assess estuarine fish species richness do not take SAR or SLR into account.

The examination of local species richness by complete census is usually not feasible (Colwell & Coddington 1994) and therefore its assessment relies on sample data. There is often a marked variability in the sampling effort applied to estuaries. In the United Kingdom, for example, estuaries like the Thames and the Severn have been intensively sampled for over 40 years (e.g. Wheeler 1979, Potter et al. 2001, Attrill & Power 2002, Colclough et al. 2002, Henderson & Bird 2010, McGoran et al. 2017), using a variety of sampling methods and resulting in more than 100 fish species being recorded in each estuary (Elliott et al. 2002, Henderson & Bird 2010). In turn, fish assemblage investigation in other UK estuaries (e.g. the Esk (E) and Lune) have only started within the last decade (Environment Agency 2017a), as prompted by the monitoring requirements of the WFD. A higher sampling effort may also be required in larger estuaries to better represent the number of species using the different habitats within the estuary. As a result, the sampling effort (as the number of the samples taken) may range over more than an order of magnitude across waterbodies and over the years (e.g. Franco et al. 2008a). This may make it difficult to disentangle the patterns of variability in the observed species richness across estuaries (e.g. SAR and SLR) from differences in sampling effort, thus creating limitations to data comparability and inclusion in the analysis (e.g. Pasquaud et al. 2015). It is generally assumed that with increased sampling then an increasing proportion of the total number of species likely to occur in an estuary will be taken. Therefore, it is expected that the cumulative number of species recorded in the samples increases with the increasing sampling effort, generating the so-called species-accumulation curve (Sanders 1968). The curve of species recorded across all samples eventually reaches an asymptote which denotes the total species complement that likely characterises an area. This assumes that even in open systems (as are estuaries) there is a finite number of species which can access the area because of their geographical and habitat/environmental preferences (although of course, global environmental factors such as climate change may cause new species to enter the species pool). Therefore we hypothesise that a species-accumulation curve can be used to estimate the species complement of estuaries.

A range of sampling methods are used for estuarine fish-based assessment, each method with its own selectivity (Franco et al. 2012, Perez-Dominguez et al. 2012). A trade-off between data standardisation (hence comparability) and representation of the full species complement of an estuary exists. On one hand, a multi-method approach, as applied for example in WFD fish-based assessment in the UK (Coates et al. 2007) is most likely to provide a more comprehensive picture of the full species complement of an estuary, although the uneven effort distribution and habitat representation of different sampling methods within an estuary may influence the comparability between estuaries. On the other hand, a single sampling method is more likely to produce a standardised approach that allows comparability between estuaries. However, the ability of the estimated species richness to represent the full species complement of any estuary may be limited to a specific part of the assemblage or type of habitat that is more efficiently sampled with the selective gear. This may introduce significant bias when comparing and contrasting species richness in geographically disparate communities sampled with different methods. In turn, this bias is likely reduced in comparative studies and assessments based on the same sampling method, albeit it must be acknowledged that in these cases and gear-specific species complement for an estuary can only be estimated. While the use of species accumulation curves is key to provide a standardised estimates of species richness (i.e. the species complement) for an area, independent of sampling effort, studies testing underlying paradigms of biodiversity such as SAR and

SLR in estuarine fish assemblages so far have only relied on observes species richness obtained from total lists of species sampled in given estuaries (Pasquaud et al. 2015, Vasconcelos et al. 2015), with the consequent limitations mentioned above. This study represented the first application of species accumulation curves (and the derived standardised estimates of the likely gear-specific maximum number of species in estuaries) to the testing of SAR and SLR. In particular, these paradigms were tested at the regional scale by using fish sample data in estuaries located between 51ºN and 56ºN latitude (England and Wales).

Materials and Methods

Biological data

From May 2006 to November 2015 inclusive, the Environment Agency and Natural Resources Wales monitored 27 estuaries across England and Wales (E&W) for WFD assessment purposes (Figure 1), using multi-gear approach (including fyke nets, seine nets, beam trawls and otter trawls). Fish species presence records obtained from the standardised use of a beach seine net (45 m by 3.5 m size, with a 5 mm knotless mesh in the centre and 20 mm mesh in the wings) have been selected for this study as this method was the only one providing the widest coverage between and within estuaries across the studied region. The estuaries were selected as a representative group of the variety of estuaries found in England and Wales (UKTAG 2006), with seine netting being undertaken in sites distributed on the lower intertidal and shallow subtidal habitats across the full salinity gradient in each estuary.

The selected dataset included a total of 3,578 samples collected at 144 sites, with the number of sites per estuary generally depending on waterbody size (Table 1). Small estuaries (<1,000ha) contained at least three to five sites, medium sized estuaries (1,000 - 10,000 ha) five to 10 sites and large estuaries (>10,000 ha) contained 10 to 12 sites. Safety and logistical constraints also influenced site selection in some cases (e.g. in the Severn, a large estuary, only five sites could be safely sampled with a seine net).

At each site, at least four samples were taken annually – two in spring (May to June) and two in autumn (September to November) given that there are seasonal migrants to estuaries (Potter et al. 2015). The number of samples taken over the period 2006-2015 in each estuary varied from 41 (Medway) to 285 (Thames) (Table 1). Explanatory variables for the SAR and SLR hypotheses were also measured for each estuary (Table 1). Specifically, waterbody size (measured as hectares (ha)), and latitude and longitude at estuary mouth (measured in degrees and decimal minutes) using ArcMap v.9.3.1, with longitude of the estuary also being recorded as a possible covariate. Additional variables characterised the estuarine conditions: mean site salinity (measured as practical salinity units) was calculated using salinity data collected by the Environment Agency between 2006 and 2015 (Graham Phillips, Environment Agency, Peterborough, Unpublished Data 2016); mean freshwater flow rates (measured as m^3 s^{-1}) over the study period for each estuary was also recorded, using data from the Environment

Agency hydrometric monitoring sites stored on the Water Information Management System (available at data.gov.uk).

Analyses

Using EstimateS (v.9.1.0), rarefaction (interpolation) curves were created for each estuarine dataset following the method for sample-based interpolation provided by Colwell et al. (2012). Species-accumulation curves were created from the cumulative number of species recorded in consecutive samples, with the sample order being randomised within each estuary dataset. A Bernoulli Product Model was used to create the rarefaction curve for each estuary, based on the mean value of 999 randomised re-runs, without replacement (i.e. each sample was selected only once). The resulting rarefaction curves provide values of cumulative species richness (*SR*) in an estuary as a function of the number of samples taken (*n*), up to the observed total species richness (SR_{obs}), as resulting from the totality of samples collected in the estuary (n_{tot}). A non-parametric estimator for species presence data, the bias-corrected form of Chao2 (Gotelli & Colwell 2011), was used to extrapolate the mean asymptotic value of the rarefaction curve, representing the maximum species richness (SR_{max}) achievable in an estuary (the gear-specific species complement). The 95% confidence interval limits (CL_{upper} and CL_{lower}) associated with the mean SR_{max} value were also calculated. In cases where the ratio of the standard deviation to the mean was >0.5, both the bias-corrected and classic forms of Chao2 method were used, and the largest of the two resulting mean SR_{max} values was selected as best estimate (Colwell 2013). To discern any potential groupings of the estuaries according to their estimated (gear-specific) fish species complement, a cluster analysis (with SIMPROF) was undertaken between estuaries based on the mean SR_{max} and the associated confidence limits. The analysis was undertaken in Primer v6.1.2, using Euclidean distance, group average cluster algorithm and 5% significance level for the SIMPROF test.

The species-area (SAR) and species-latitude (SLR) paradigm hypotheses were tested using generalised additive models (GAMs). Estuary size and latitude were used as explanatory variables for SR_{max} and longitude was also included as a possible covariate. The small size of the dataset (27 estuaries) prevented the inclusion of all three variables in a single model, and therefore a modelling strategy was adopted whereby three models were generated including all possible combinations of pairs of the three variables (*m1* with size and latitude as predictors, *m2* with latitude and longitude, *m3* with size and longitude) to account for possible combined effects. GAM modelling was undertaken using the mgcv package in R (Wood 2006, R Core Team 2017), with the following parameters specified: negative binomial family (with log link function); thin plate regression splines as smoothing functions for all explanatory variables (with default basis dimension k = 10, except for latitude in *m2*, where k was set to 18, the maximum value for k allowed by the dataset size); an additional penalty added on the null space of the original penalty for all covariates (select = TRUE); and REML used as smoothness selection method. Model diagnostic was undertaken (checking of residuals for assumptions, overfitting and overdispersion) to assess the validity of the models. The significance of the model predictors was assessed based on model summary results, and the deviance component explained by each individual predictor in the model was assessed as an indicator of the magnitude of the effect, by comparing nested models (i.e. *m1*, *m2* and *m3* against models calibrated for individual variables using the same model parameters (as described above) using hypothesis testing (*anova.gam*).

Results

Fish assemblage composition

Across all estuaries in the study, 114 species were recorded (Supplementary Material A1). The total observed species richness ranged from 22 (Esk(E)) to 55 (Carrick Roads Inner; Dart) with a mean of 35.3 (SD=10.1) (Table 1). Five of the 114 species were encountered in every estuary (*Platichthys flesus* (flounder), *Pleuronectes platessa* (European plaice), *Pomatoschistus microps* (common goby), *Pomatoschistus minutus* (sand goby); *Sprattus sprattus* (European sprat)) and 20 were recorded in only one estuary (Supplementary Material A1). The taxa were listed per estuary and following Franco et al. (2008b), were categorised into one of six Estuarine Use Functional Guilds, based upon the way that the species use an estuary (Supplementary Material A1). Thirty two of the 114 species recorded in this study, are classified into more than one category by Franco et al. (2008b). Two are considered catadromous (European eel (*Anguilla anguilla*) and thin lipped grey mullet (*Liza ramada*), with the thin lipped grey mullet also considered a marine migrant in some estuaries. Seven are anadromous with the three-spined stickleback (*Gasterosteus aculeatus*) and sea trout (*Salmo trutta*) being the most frequently encountered in estuaries across the study area. Twenty are categorised as estuarine species (common goby (*Pomatoschistus microps*) and sand goby (*Pomatoschistus minutus*) caught most frequently in this group; 27 estuaries)). Twenty four freshwater species encountered in the study, the most common of which were including roach (*Rutilus rutilus*), Eurasian minnow (*Phoxinus phoxinus*) and common dace (*Leuciscus leuciscus*).

Of the 15 marine migrants encountered in the study, flounder (*Platichthys flesus*), European plaice (*Pleuronectes platessa*) and European sprat (*Sprattus sprattus*) were caught in all estuaries in the study although flounder is also regarded as semi-catadromous given that it spends most of its time in estuaries after breeding at sea (Potter et al. 2015). The most numerous category of fishes in the study was marine stragglers, with 31 species caught in the study area. The longspined sea scorpion (*Taurulus bubalis*) and greater sand eel (*Hyperoplus lanceolatus*) are the most frequent, caught in 16 and 15 estuaries respectively.

Forty species were consistently present in at least 90% of the samples taken per estuary (Supplementary Material A1) thus characterising the dominant assemblage for each estuary for the geographical area covered by this study. Per estuary, either 11 or 12 species were caught in ≥90 % of samples, apart from the Severn, with 16 species listed. The common goby, sand goby (*Pomatoschistus minutus*) and

European sprat were consistently caught in 26 of the 27 estuaries, with European flounder being the only species that was caught in ≥90% of the samples in every estuary.

Two species of the wrasse family, corkwing wrasse (*Crenilabrus melops*) and ballan wrasse (*Labrus bergylta*), were caught consistently in the South West of the study area (Carrick Roads Inner and Dart, respectively). Three species of sandeels were consistently recorded (small sandeel (*Ammodytes tobianus*), Corbin's sandeel (*Hyperoplus immaculatus*) and great sandeel (*Hyperoplus lanceolatus*)) and three clupeids were also present (herring (*Clupea harengus*), European pilchard (*Sardina pilchardus*) and European sprat), as were four cyprinids (common bream (*Abramis brama*), common dace (*Leuciscus leuciscus*), Eurasian minnow (*Phoxinus phoxinus*) and roach (*Rutilus rutilus*)). Three gadoids were caught consistently (Atlantic cod (*Gadus morhua*), whiting (*Merlangius merlangus*) and pollack (*Pollachius pollachius*)) and five gobies (black goby (*Gobius niger*), two spotted goby (*Gobiusculus flavescens*), common goby, sand goby and painted goby (*Pomatoschistus pictus*)).

Species-accumulation curves

The species rarefaction curves are similar in overall shape for each estuary, with the first 50 samples providing the steepest part of the species accumulation (Figure 2). Three of the 27 estuaries have over 50 or more species recorded (Carrick Roads Inner, Dart and Dee), two of which reach over 50 species within 100 samples (Carrick Roads Inner, Dart).

Some estuaries, such as the Taw/Torridge and the Thames, have a pronounced profile of a steep gradient in the first 50 samples with the curve quickly levelling off thereafter. The Thames is the most highly sampled estuary in the dataset, with a total of 285 samples, yet few species are caught (SR_{obs}=34). When n=50, SR=24 i.e.71 % of total observed number of species is detected within 18% of the samples collected, with the remaining ten species being recorded over the next 235 samples. The profile of other estuaries such as the Severn and Southampton Water, have a less pronounced levelling off phase. With Southampton Water, when n=50, SR=26 (63% of total observed species richness with 21% of samples) with the remaining species being recorded over the further 186 samples. This suggests that not only is Southampton Water recording more species (41 compared to 34 for the Thames) but also the recorded species are more evenly spread throughout all the samples, thus requiring more effort to gain an understanding of the entire species composition that can be sampled with the seine net. The steep profile of the Severn is exacerbated by the low number of seine net samples (n=48) collected in this estuary over the studied period.

3.3 Estimated maximum species richness

SR_{max} calculated for the studied estuarine fish assemblages (as sampled by seine net) ranged from 24.39 (Medway) to 73.97 (Dart); with an overall mean of 42.08 species (SD = 12.54) (Table 2). The total percentage of sampled species compared to the estimate of asymptotic species richness (SR_{obs}/SR_{max})

that could be caught by seine netting in the studied estuaries ranges from 55 % (Tweed) to 100% (Taw/Torridge).

SAR and SER hypothesis testing

The three models calibrated to test the SAR and SLR hypotheses explain respectively 68.7% (*m1*, size and latitude as predictors), 57.5% (*m2*, latitude and longitude), and 19.5% (*m3*, size and longitude) of the total deviance in SR_{max} data. Latitude always results as a highly significant predictor in all models where this variable is included (*m1* and *m2*). Both models indicate a net decrease in species richness with increasing latitude, this decrease being particularly marked between 50°N and 52°N (Figure 3). Some fluctuations (secondary maxima) can be observed at latitudes around 53°N and 56°N due to the higher species richness recorded in the Dee, Humber and Tweed compared to other estuaries at similar latitudes (Table 2). Estuary size is also a significant predictor, albeit only when coupled with the latitudinal effect in *m1*, with the species richness increasing with increasing estuary size (Figure 3). The latitudinal effect is in general larger than the size effect, as indicated by the deviance explained by each of these predictors in the models (Figure 3).

Estuary groupings

According to the classification analysis (cluster and SIMPROF) based on SR_{max} data (mean and confidence limits), a group of five estuaries (Tweed, Dee, Poole Harbour, Dart and Southampton Waters; Table 2, Figure 4) significantly ($P < 0.05$) differentiates from the others due to the general higher mean SR_{max} values (ranging 46 to 74, > 60 in most cases, overall group mean of 61), albeit the highest uncertainty was associated with these mean estimates (confidence limit interval between 37 and 81, 57 on average). The estuaries in this group are of variable size (from 244 to 10,928 ha, 3,681 ha on average) and are located between 50.4°N and 55.7°N of latitude (52.2°N on average).

The remaining 22 estuaries have variable mean SR_{max} values, between 23 and 59 (mostly < 50), and they further differentiate ($P < 0.05$) into four groups (A1-A4; Table 2, Figure 4). Group A1 comprises of seven estuaries (Medway, Esk, Telfi, Lune, Tees and Taw/Torridge) of small/medium size (28 to 5,657 ha, 1,406 ha on average) and located between 51.1°N and 54.6°N latitude (53.1°N. on average). These estuaries have the lowest mean SR_{max} (always < 34, 29 group average) compared to the other estuaries, with the highest confidence associated with these estimates (confidence limit interval of 14 species on average, generally <26) . Groups A2 and A3, each comprised of six estuaries (Table 2), have intermediate values of mean SR_{max} (mostly around 40, ranging 32 to 46 overall). However, the uncertainty around these mean estimates differs between the two groups, being higher in A2 (confidence limit interval of 16 species on average) and lower in A3 (confidence limit interval of 40 species on average). Estuaries from these two are located at latitudes between 50.5°N and 53.7°N (with an average value close to 52°N in both groups), and most of these estuaries are of medium size (around 1500 ha), with the notable presence of one large estuary in each group (Thames in A2 and Severn in A3). Group A4 is only comprised of three estuaries (Exe, Humber and Carrick Roads Inner) that are of medium to large size (1259 ha to 34647 ha, 11900 ha on average) and are located at a lower latitude than the others, on average (51.5°N, ranging 50.2°N to 53.7°N). These estuaries have higher mean SR_{max} values (between 49 and 59, 53 on average), with a relatively low uncertainty associated (confidence limit interval of 19 species on average).

Discussion

Fish species complement of estuaries, SAR and SLR paradigms, and other possible influencing factors

Examining the relationships of localised assemblages from varied study areas and effort using rarefaction curves has proved successful in tree and insect studies (Colwell et al. 2012) although this approach has been rarely used for fishes. Quantifying biodiversity using rarefaction curves and asymptotic estimators is a method not often used for estuarine fish assemblages at a regional scale. Most of the studies investigating fish species richness in estuaries and their patterns in relation to natural and/or anthropogenic variability are based on surveys that are assumed to be a complete census of a localised assemblage, without necessarily considering the implications of varying sample effort and/or methods on the completeness of the assemblage that has been measured (Franco et al. 2008b, Nicolas 2010a, Nicolas 2010b, Vasconcelos et al. 2015).

In the present study, before any hypotheses were examined, an objective examination of the effectiveness of the sampling to obtain a species census was firstly undertaken by estimating the maximum number of species in each estuary that can be caught using a seine net. The estimator used in this study for the sample-based incidence data is well proven in a variety of ecological fields (Chao 1987, Shen et al. 2003, Gotelli & Colwell 2011, Chao et al. 2015). We acknowledge that the approach we used in this paper is not free from limitations. The purpose of applying rarefaction curves in our study was to obtain standardised species richness data to allow comparing and contrasting between estuaries and as such the ability to examine and explore the SAR and SLR paradigms set out in this paper. Therefore, we chose to select fish data from a single sampling method (seine netting) that has been used in a consistent and standardised way across the studied estuaries, to allow a better control of the effects of sampling variability and effort. As a result, the approach applied in this paper cannot be considered to be a complete census of the fish assemblage present in an estuary, due to the limitations imposed by the selectivity, efficiency and habitat sampled with the selected method. The calculation of estimated total species richness is bounded by this method and, as such, must be considered as a gear-specific indicator of the fish species complement of an estuary. Although, in absolute terms, the resulting estimates may differ compared to the known species richness from other studies using multiple or different sampling methods (as discussed in detail further below), we are more confident that the standardised estimates we used allow us to do a more robust comparison between estuaries, while controlling for the effects of sampling variability and effort.

The latitudinal gradient in diversity has been examined for over two centuries and the attenuation of species diversity as one travels further from the equator has been recorded by multiple authors examining many biota and regions (Jablonski et al. 2017). However the causal processes that drive this phenomenon remain elusive (Hillebrand 2004). Several hypotheses have been proposed to explain this phenomenon, which Brown (2014) has grouped into three main categories: phylogenetic niche conservatism, ecological productivity and kinetics.

Wiens & Donoghue (2004) considered that species' ancestral niches were tropical, preventing wide-scale adaptation to temperate niches in recent history due to factors such as glaciation. In the case of the study area, this phylogenetic niche conservatism hypothesis is not considered to be a causal process for the latitudinal pattern observed in this study. The northern estuaries were covered by an ice sheet for longer although the interconnected nature of the UK waters would suggest that this is no longer a factor 11,000 years after the last glaciation.

An ecotone, representing a change in ecological productivity, is the boundary between biogeographic regimes where there is merging of two adjacent assemblages and so the ecotone has elements of both assemblages and thus can be richer than either of the merged elements (Basset et al. 2013). In the case of the British Isles, the influence of the North Atlantic Drift especially on south-western areas exacerbates the mixing of Boreal and Lusitanian faunae (Henderson & Henderson 2017). Therefore it would be valuable in the future to categorise the estuarine fish assemblage members according to their Boreal and Lusitanian origins to show where the warmer Lusitanian fauna from the Iberian Peninsula merges with the colder Boreal community from NW Europe and the North Sea (Wheeler 1969). Furthermore, there is a depth effect between the shallow North Sea to the East compared to the deep waters off the coastal shelf to the West suggest that a longitudinal element would have some effect on patterns of diversity in this regional study, as has been previously reported (Nicolas 2010b). However, the inclusion of longitude in the models in the present study do not support relationships between longitude and species diversity, or a combination of longitude with latitude and species diversity (see below).

By detailing the observed latitudinal gradient in many biological realms, Fischer (1960) reviewed studies detailing the observed latitudinal gradient in many biological realms and concluded that this phenomenon is illustrated best in the marine field and that climates with higher and consistent temperatures support higher diversity. Brown (2014) notes that greater rates of metabolism, ecological dynamics and coevolutionary processes are all supported by higher temperatures. In the context of estuarine fish ecology, higher temperatures at lower latitudes, leading to higher biological rates have also been suggested as leading to biogeographic differences (Henriques et al. 2017), perhaps due to shorter generation times and higher mutation rates (Gaston 2007). This kinetic argument is considered to be the most likely cause of the latitudinal gradient shown here. Multiple agencies across the marine field now record extensive thermal measurements in inshore waters. The diversity-temperature relationship could be further explored by integrating existing temperature records with this biological dataset. In supporting the SER of species richness and latitude, this study suggests that increases in sea temperatures as a result of climate change could increase diversity in estuarine fish species richness in temperate waters (Attrill & Power 2002, Henderson 2007, Hiddink & Hofstede 2008, Robins et al. 2016). This may also result in increased abundance although density-dependence has been shown to be a limiting factor on the abundance of sprat in the Bristol Channel (Henderson & Henderson 2017).

The species-area relationship also proved significant although the effect was only noticeable when combined with latitude. This is in contrast to previous studies which have found the relationship between size and estuarine fish diversity to be highly significant (Harrison & Whitfield 2006, Franco et al. 2008a, Nicolas et al. 2010a). It is assumed that a larger sample area would contain more individuals as well as more species (Whittaker & Fernández-Palacios 2007) perhaps due to habitat heterogeneity opportunities over a larger area (Báldi 2008).

At the global extent, Vasconcelos et al. (2015) found that species richness of marine fish correlated highly with latitude, with estuary size being only important at the regional extent. This study indicates that estuary size alone is not sufficient as a driving influence on species richness. Of the three estuaries with surface areas greater than 20000 ha (Humber, Thames and Severn), the Humber is the only estuary that records high diversity with either observed species or predicted total richness. The high diversity measured in the Humber cannot be explained by high heterogeneity as the Humber contains as many large-scale habitats as the Thames and many fewer than the Severn (JNCC 2015).

Unlike the Humber, the Thames has few observed species of both marine guilds and estuarine species and therefore the overall species richness is comparatively poorer. The Thames, despite its southerly latitude, has a relatively narrow shelf providing few marine species to the assemblage and even then the uniform sedimentary habitats of the southern North Sea create fewer niches and thus species (Ducrotoy et al. 2000). By classifying the habitat attractiveness for fishes in an estuary, Amorin et al. (2017) noted potential changes to the functioning of the fish community and the nursery carrying capacity over time. A similar approach could be used spatially with this dataset to further investigate the relationships between habitat types and fish communities in the Thames and the rest of the estuaries in the study area. The Thames only started to regain its estuarine fish community in the 1960s after many decades of being abiotic (Elliott & Hemingway 2006, Taylor 2015, Henderson 2017). Furthermore, the Thames has been subject to severe and sustained environmental degradation (Coates et al. 2007), notably habitat loss particularly in the mid and upper reaches of the estuary and the presence of a water quality barrier due to low dissolved oxygen, and these may have contributed to reduce the species richness in this estuary. Significant pollution events continue in the Thames catchment (Environment Agency 2017b).

By further exploring the relationship between species richness drivers such as habitat functioning as well as anthropogenic factors such as pollution events, it may be possible to further explain the pattern of differentiation between not only the Thames assemblage but also the estuarine populations described in this paper.

The historical sampling of the Severn Estuary fish assemblage gives the opportunity to validate the analysis in this paper. This estuary is considered to be one of the most diverse estuaries in the UK (Potts & Swaby 1993) and was designated under the Ramsar Convention in 1995 (JNCC 2008). Only 27 species recorded in the Severn in this study , and this show the influence of both differing sampling methods and a greater sampling effort in the previous studies. Using once-monthly power station sampling at the edge of a large intertidal mud flat, in the greater Severn estuary, Henderson & Bird (2010) recorded a total of 83 species over 28 years, with a notable predominance of species of marine origin in the assemblage (77% of the species), compared to the present study (59%). While SR_{max} is estimated to be much higher than SR_{obs} for the Severn estuary, the SR_{max} value predicted in this study (38) is still far lower than the 83 species recorded by Henderson & Bird (2010). This is probably the result of the intense nature of power station sampling compared to seine netting. Therefore while further seine net sampling is expected to reveal more species, the nature of the method is not expected to yield similar numbers of taxa as Henderson & Bird (2010) or Potts & Swaby (1993).

Despite the Tweed being the most northerly and one of the smallest estuaries in the study, it has one of the highest estimated maximum species richness values (46). However, a high uncertainty is associated with this estimate, as attested by the large confidence interval (the largest of all the assessed estuaries), suggesting caution needs to be applied when drawing conclusions regarding its estimated maximum value. Continued sampling may help to increase confidence in the overall assessment. In terms of species presence in estuaries, there are only a few species adapted to the life in changing environments as estuaries (and these are highly abundant, confirming the stress-subsidy continuum) (see below). Most of the species occurring in estuaries have been found to be transient species, either migratory species or stragglers (Franco et al. 2008a), with most of the contribution to species diversity coming from the marine realm rather than from freshwaters (Whitfield et al. 2012). The dominance of marine taxa as a proportion of the overall fish species richness of an estuary is well defined (Potter et al. 1990, 2015, Pease 1999, Whitfield 1999) and is consistent throughout the study area and the current estuarine datasets present, with some notable exceptions. Categorising the marine species into those that generally inhabit coastal areas and only enter estuaries accidentally and in low numbers (marine stragglers) and those that often spawn at sea and enter estuaries in high numbers, particularly as juveniles in defined patterns (marine migrants), aids understanding of both natural and anthropogenic impacts on estuaries (Elliott et al. 2007b).

In accordance with the literature, a higher proportion of marine straggler species appears to characterise the estuaries where a higher overall species diversity was estimated in this study (e.g. Tweed, Dee, Poole Harbour, Dart and Southampton Waters). The width of the estuary mouth has been shown to be an important predictor of species richness, particularly marine species, in previous studies (Pease 1999, Roy et al. 2001, Nicolas et al. 2010b, Tweedley et al. 2017). However, the high diversity estuaries mentioned above do not show particularly large mouths compared to other estuaries with less diverse fish assemblages (as for example the Severn). We argue that it is the mouth width-to-estuary size (e.g. area) ratio rather than the mouth width in itself that affects the predominance of marine species occurring in the estuary as a whole, as this not only accounts for the accessibility of the estuary to species entering from the adjacent marine area, but also the penetration of these species into the estuary and their distribution across estuarine habitats (likely to be enhanced where the mouth-to-estuary size ratio is higher, resulting in the estuary resembling more a marine embayment). This argument appears to be supported by the findings by Perez-Ruzafa et al. (2007) on the hydrographic and geomorphologic determinants of fish assemblages in coastal lagoons. These authors found that a morphometric parameter (named restriction ratio) measuring the ratio between the width of the lagoon entrances and the lagoon perimeter (a proxy for the waterbody size) was amongst the primary constraints affecting the fish assemblage composition in the lagoons, mainly through influences on the temperature and salinity regime of these systems. The latter factor is of particular relevance to the entrance and penetration of estuaries by stenohaline marine species as are marine stragglers.

The presence of some straggler species permeating throughout certain estuaries and their presence in nearly all samples collected across those estuaries challenges the expected biological preferences of those species, or their functional categorisation for the estuaries within this study. Exploring those estuaries that are of particular significance to the unexpected 'generalists' with extended sampling may explain how these species adapt and thrive across the highly heterogeneous and challenging estuarine environment.

The above feature reflects the so-called stress-subsidy continuum, whereby variable conditions in estuaries are stressful for those species not adapted to them but a subsidy for those that are adapted (Elliott & Quintino 2007). For example, there are some ubiquitous and euryecious species such as the European flounder (*Platichthys flesus*) (Borg et al. 2014, Vinagre et al. 2005) and its presence in all areas of the 27 estuaries in this study underlines its importance to estuarine fish assemblages. It has been noted however that there can be changes even to this species due to both natural and anthropogenic factors (Amorim et al. 2017), with a major decrease in European flounder recorded in a Portuguese estuary (Cabral & Costa 1999), possibly due to climate change (Cabral et al. 2001).

A notable exception to the patterns mentioned above is the high fish diversity observed and estimated in this study for the Humber, which results from a particularly high number of freshwater taxa. The high percentage of freshwater taxa in the Humber may be due to the large catchment and high fluvial flow, resulting in low overall site salinity despite the sites being located in the oligohaline, mesohaline and polyhaline areas, allowing freshwater taxa to actively or passively occur in greater numbers into the estuary.

The influence of a latitudinal-longitudinal combination factor (i.e. SW to NE) rather than either on its own is expected to be important in the context of the British Isles given that the SW has the larger influence of the warmer waters of the North Atlantic Drift and the NE the influence of colder North Sea waters (Nicolas 2010b). If the estuarine fauna was therefore mainly the result of the influence of its shelf components (the marine migrants and the straggler species) then this would have a dominating effect, as has been found previously (Vasconcelos et al. 2015). Accordingly, the main influence would be a gradient from the SW to the NE of the study area but longitude was not a significant explanatory variable of species richness in our study.

Implications for monitoring and management

The size and nature of the full fish species complement of an estuary are regarded as indications of the ecological status and so management measures are required if that status falls below what is expected. This is the central raison d' être of determining Good Ecological Status under the EU Water Framework Directive (Hering et al. 2010). The determination of the asymptote and the number of samples required to achieve that is therefore important for managers who have to allocate sufficient resources to quantify and understand the ecological status of an estuary.

Examination of the rarefaction curves suggest that in most estuaries, most of the species richness (that can be sampled with a seine net) is achieved within 100 samples, beyond which continued sampling provides relatively additional taxa. This analysis not only shows what proportion of the assemblage has been encountered with the available sampling but it can be used proactively to define the field methods to help managers understand when continued and further sampling is required. As mentioned before, each method for monitoring fishes in estuaries will take a slightly different component of the assemblage and several methods are needed concurrently in order to take all species (Elliott et al. 2002). The WFD requires using the fish species complement is a predominant factor and metric in determining the health and ecological status of an estuary (Coates et al. 2007). It is therefore emphasised that multi-gear surveys provide an effective way to reach the full species complement. However due to the heterogeneous and harsh nature of estuarine environments, it is difficult to obtain the entire species complement and so such a survey is not cost-effective.

The current study suggests that regional classification tools, such as those aimed at ecological status assessment (WFD 2000/60/EC), that do not take latitude and estuary size into account may misrepresent the anthropogenic influences on estuaries as species richness decreases with latitude, and, in certain conditions, increases with size, irrespective of anthropogenic impact (acknowledging the variable impacts across the estuaries presented in this study).

Through the driver of the WFD, competent authorities now have extensive information on the hydromorphological attributes of estuaries, including the width of the estuary mouth. Coupled with the ever-increasing biological data, it is recommended that the complex interactions are explored to determine if any factors beyond SLR and SAR influence fish diversity in temperate estuaries.

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Tables

Table 1 Total species caught (SR_{obs}), site number (n_{sites}) and sample number collected per estuary (n) during surveys from 2006 to 2015 in selected estuaries. Waterbody size, latitude and longitude. Mean site salinity and mean river flows measured over study period.

Estuary	SR_{obs}	n_{sites}	n	Size (ha)	Latitude	Longitude	Mean site salinity (psu)	Mean fluvial flow (m3/s)
Adur	31	5	114	137	50.85	-0.28	15.40	0.35
Alde & Ore	25	4	52	1088	52.12	1.54	29.25	0.89
Camel	45	7	156	1091	50.55	-4.91	25.71	0.74
Carrick Roads Inner	55	7	147	1259	50.21	-5.04	28.86	1.25
Conwy	37	3	111	1557	53.29	-3.84	23.33	22.82
Dart	55	5	125	831	50.38	-3.60	20.40	13.41
Dee	52	9	215	10928	53.32	-3.19	14.89	43.26
Esk(E)	22	4	97	28	54.48	-0.61	20.50	5.79
Exe	42	5	89	1793	50.63	-3.44	23.40	26.55
Foryd Bay	36	3	100	243	53.11	-4.32	32.33	6.17
Humber	48	12	237	32647	53.71	-0.48	8.42	139.28
Lune	25	2	76	302	54.02	-2.83	15.00	73.20
Medway	23	3	41	5657	51.41	0.64	21.33	12.59
Milford Haven Inner	34	7	256	2102	51.72	-4.91	24.00	15.18
Nyfer	25	3	107	103	52.02	-4.84	29.00	35.92
Orwell	38	4	139	1249	52.00	1.23	31.25	1.49
Poole Harbour	49	11	180	3309	50.70	-2.00	24.18	4.64
Ribble	34	4	84	4528	53.71	-2.97	13.75	39.80
Severn	27	5	48	53645	51.81	-2.54	13.60	127.03
Southampton Water	41	8	236	3091	50.87	-1.36	26.88	21.69
Stour	36	5	127	2553	51.95	1.18	30.20	0.97
Taw/Torridge	32	8	171	1461	51.07	-4.16	21.63	37.28
Tees	27	2	74	1143	54.62	-1.18	26.00	27.20
Teifi	26	3	75	616	52.11	-4.69	13.33	35.92
Thames	34	8	285	24842	51.49	0.25	7.63	82.59
Tweed	25	5	123	244	55.76	-2.04	9.20	13.90
Wyre	29	2	44	637	53.88	-2.98	18.00	8.71
Min	22	2	41	28	50.21	-5.04	7.63	0.35
Mean	35.3	5	130.0	5818	52.29	-2.27	21.02	29.58
Max	55	12	285	53645	55.76	1.54	32.33	139.28
SD	10.1	3	67.7	12195	1.50	2.11	7.29	36.63

Table 2 Abbreviated estuary name, estimated maximum species richness (*SR_{max}*) with 95% confidence limits (CL). Groupings as identified by the cluster analysis are also reported.

Estuary	Abbreviated Name	SR_{max}	CL_{lower}	CL_{upper}	Group
Medway	Medway	23.37	23.03	28	A1
Esk(E)	Esk(E)	25.71	22.63	43.82	A1
Teifi	Teifi	26.49	26.04	32.15	A1
Lune	Lune	30.18	25.98	52.28	A1
Tees	Tees	30.45	27.64	45.67	A1
Taw/Torridge	Taw/T.	32.14	32.01	35.23	A1
Wyre	Wyre	33.4	30.01	48.24	A1
Thames	Thames	37.32	34.5	56	A2
Stour	Stour	37.86	36.29	47.91	A2
Foryd Bay	ForydB.	39.47	36.64	54.73	A2
Orwell	Orwell	41.47	38.69	55.46	A2
Conwy	Conwy	41.62	37.94	59.65	A2
Camel	Camel	45.85	45.09	52.93	A2
Alde & Ore	Al&Or	32.36	26.3	66.77	A3
Nyfer	Nyfer	34.91	26.86	77.68	A3
Severn	Severn	37.77	29.82	68.14	A3
Adur	Adur	37.94	32.33	67.25	A3
Milford Haven Inner	MH Inn.	41.17	35.59	66.26	A3
Ribble	Ribble	45.12	36.62	81.19	A3
Exe	Exe	48.8	43.69	69.38	A4
Humber	Humber	50.49	48.41	63.22	A4
Carrick Roads Inner	C.R.Inn	58.97	55.85	73.52	A4
Tweed	Tweed	45.83	30.04	111.08	B
Southampton Water	SotonW.	60.42	46.19	113.62	B
Dee	Dee	60.96	54.12	89.86	B
Poole Harbour	PooleH.	62.13	52.42	99.34	B
Dart	Dart	73.97	61.1	113.97	B

Figures

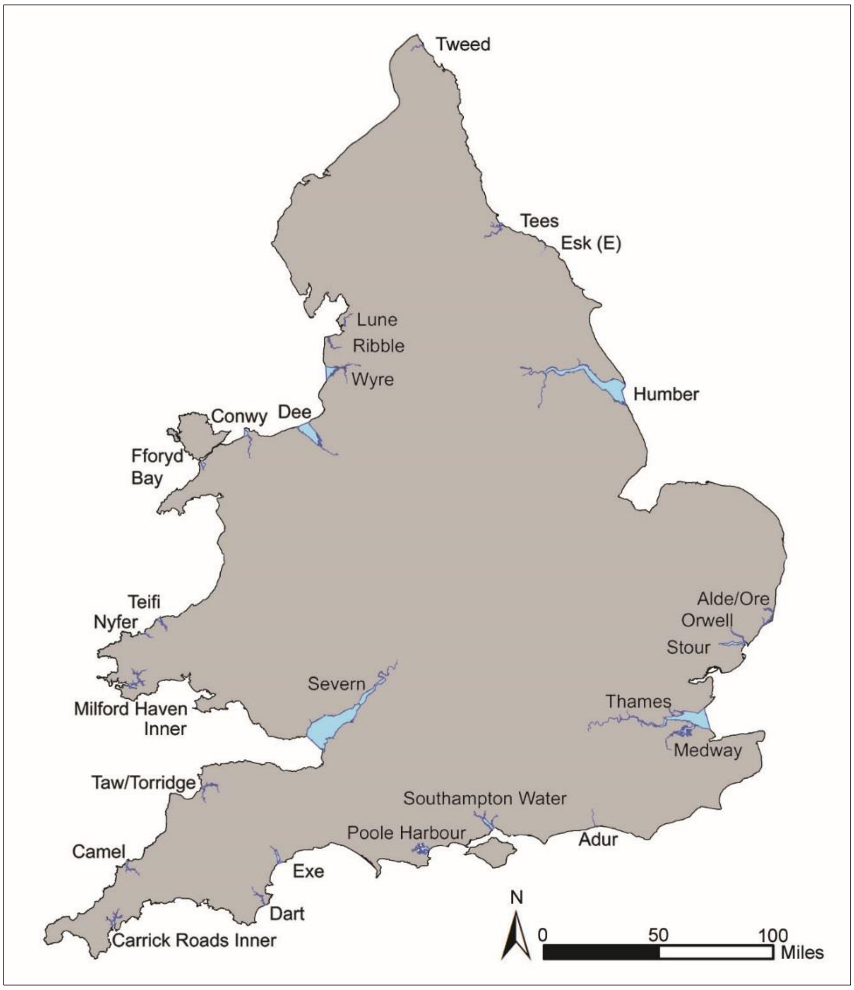

Figure 1 Estuaries monitored in present study

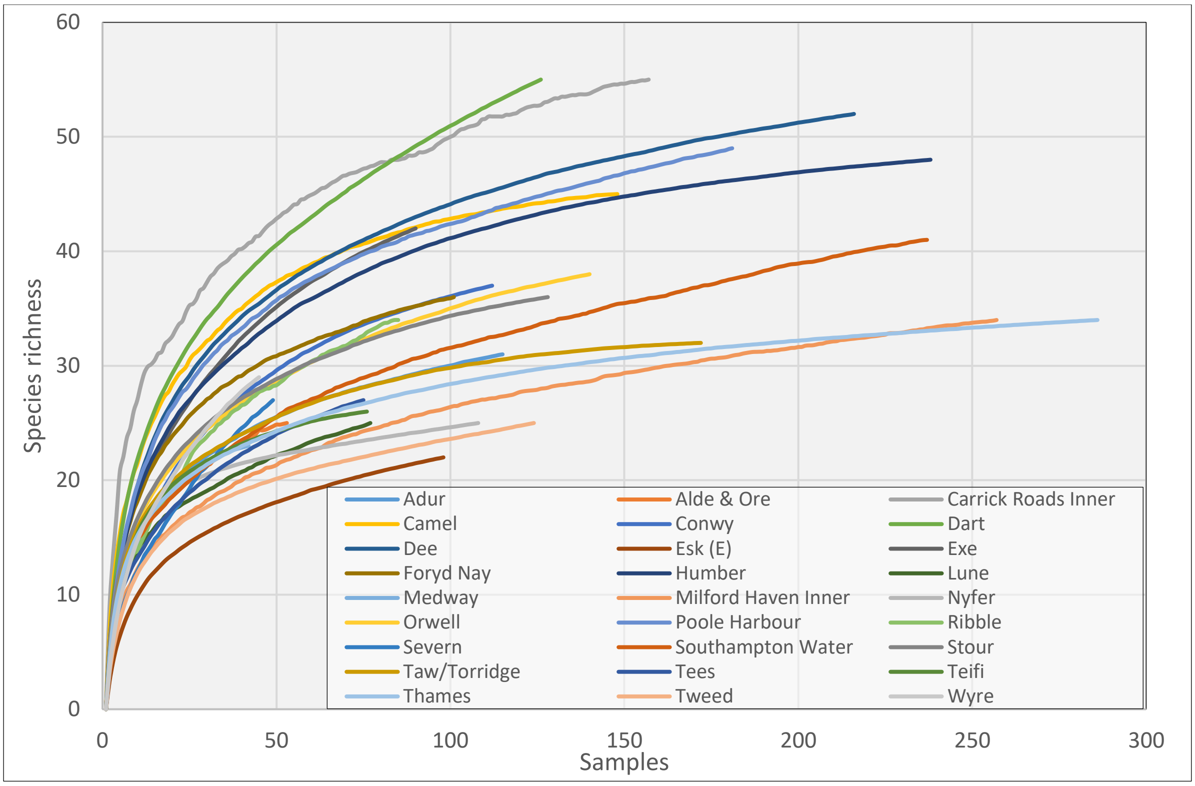

Figure 2 Rarefaction curve for pooled data per estuary. Each curve represents the mean of up to 999 randomisations.

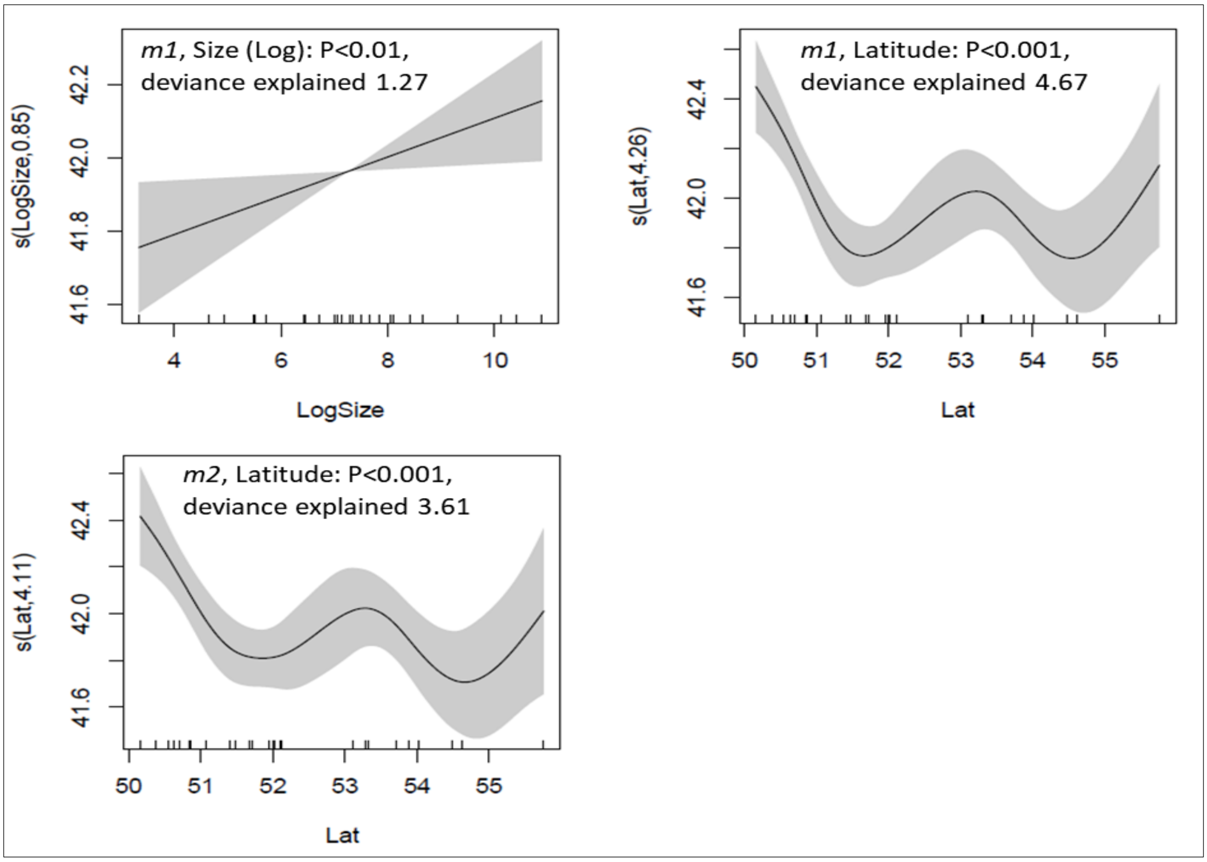

Figure 3 GAM smoothing curves for significant predictors of SR_{max}, with associated confidence interval (shaded area). Significance and magnitude of the effect (deviance explained by the individual predictor in the model) are indicated. Curves have been rescaled to reflect variability on the SR_{max} scale.

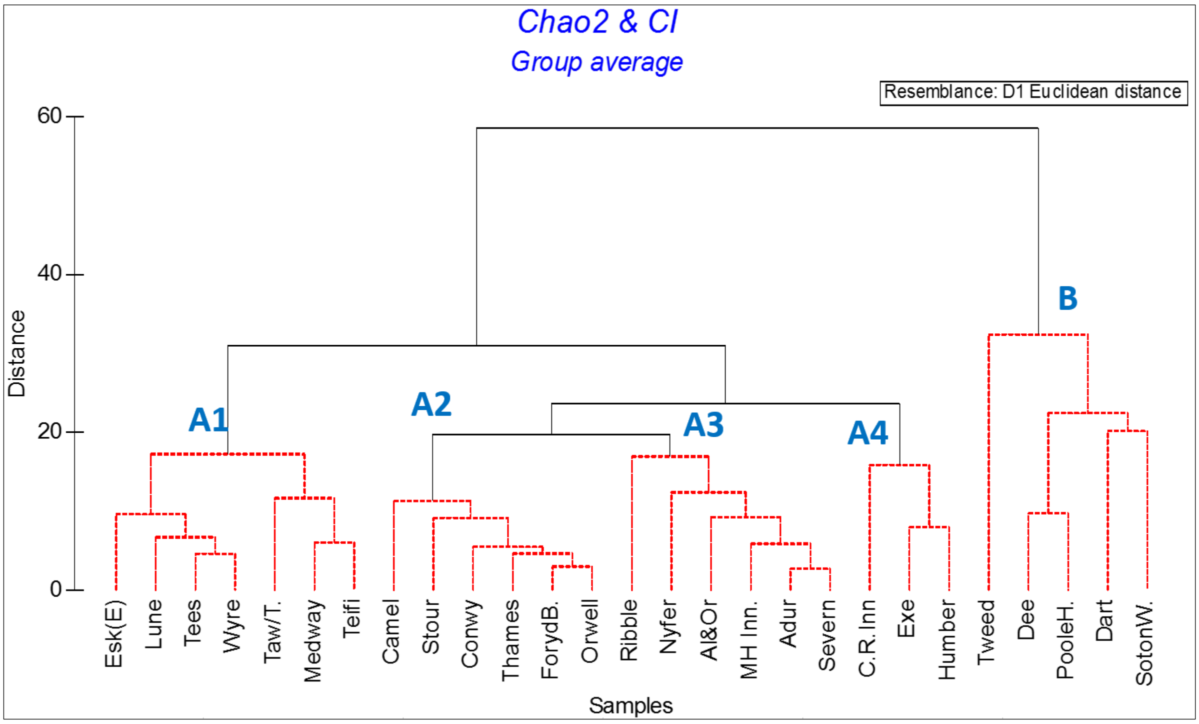

Figure 4 Cluster analysis of the studied estuaries based on SR_{max} estimates (mean and confidence limits). Significantly different groups (SIMPROF, P < 0.05) are indicated with solid black lines. Groupings are indicated (A1-B).

Supplementary Material A1 Observed taxa over study period per estuary. Categorised into one of six Estuarine Use Functional Guilds (EUFG) (Franco et al. 2008). Single asterisk denotes presence. Double asterisk denotes taxa present in estuary in at least 90 percent of all samples. Total number of taxa per estuary and total number of taxa present in at least 90 percent of all samples per estuary noted at bottom of table.

Latin Name	Common Name	EUFG	Adur	Alde & Ore	Carrick Roads Inner	Camel	Conwy	Dart	Dee (N_Wales)	Esk (E)	Exe	Foryd Bay	Humber	Lune	Medway	Milford Haven Inner	Nyfer	Orwell	Poole Harbour	Ribble	Severn	Southampton Water
Abramis bjoerkna	Silver bream	F	*										*									
Abramis brama	Common bream	F	*					*					**									*
Agonus cataphractus	Hooknose	ES, MS				*	*	*	*		*	*	*			*				*		
Alburnus alburnus	Bleak	F											*									
Alosa alosa	Allis shad	A				*																
Alosa fallax	Twaite shad	A		*																	*	
Ammodytes tobianus	Small sandeel	ES, MS	**		*	**	**	*	**	**	**	**	*	*		*	**	*	*	**	**	
Anguilla anguilla	European eel	C	*	*	*	*	*	*	*		*		*	*	*	*	*	*	*	*	**	*
Aphia minuta	Transparent goby	ES, MM		*	*			*	*						*			*	*		*	*
Apletodon dentatus	Smallheaded clingfish	MS			*			*														
Atherina boyeri	Sandsmelt	ES																				
Atherina presbyter	Sandsmelt	MM	**	**	**	**	**	**	*	*	*	**	*		**	**	**	**	**	*	*	**
Barbatula barbatula	Stone loach	F												*								
Belone belone	Garpike	MM, MS			*	*	*	*			*				*	*			*			*
Buglossidium luteum	Solenette	MS	*		*	*			*		*	*			*				*			*
Callionymus lyra	Dragonet	MS		*	*	*		*	*		*		*					*	*	*		*
Callionymus reticulatus	Reticulated dragonet	MS																*		*		*
Carassius auratus	Goldfish varieties	F	*																			
Chelidonichthys lucernus	Tub gurnard	MM, MS			*	*			*		*	*							*		*	
Chelon labrosus	Thicklip grey mullet	MM	**	*	**	**	*	*	*		*	*			*	**	**	**	*		*	**
Ciliata mustela	Fivebeard rockling	MM			*	*	*	*	*		*		*					*	*		*	*
Clupea harengus	Herring	MM	**	**	*	**		**	*	**	**		**	**	**	**	**	**	**	**	**	**
Cobitis taenia	Spined loach	FS											*									
Conger conger	Conger eel	MS							*													
Cottus gobio	Bullhead	F												*						*		
Crenilabrus melops	Corkwing wrasse	MS			**	*		*			*	*						*	*			*
Crystallogobius linearis	Crystal goby	MS							*												*	

Ctenolabrus rupestris	Goldsinny	MS			*			*														
Cyclopterus lumpus	Lumpsucker	MM, MS																				
Cyprinus carpio	Common carp	F																				
Dicentrarchus labrax	European seabass	MM	**	**	**	**	**	**	*		**	**	*	*	**	**	**	**	**	**	**	**
Echiichthys vipera	Lesser weever	MS				*	*		**		*	*	**							*		
Engraulis encrasicolus	European anchovy	MM, MS					*	*	*		*				*							
Entelurus aequoreus	Snake pipefish	MS		*	*	*		*					*			*		*	*			
Esox lucius	Pike varieties	F							*				*									
Eutrigla gurnardus	Grey gurnard	MM, MS					*		*													
Gadus morhua	Atlantic cod	MM					*	*	*	*		*	*	*			*			*	**	
Gaidropsarus mediterraneus	Shore rockling	MS						*												*		
Gasterosteus aculeatus	Threespined stickleback	A, ES, F	*	**		*	*	*	**	**	*	**	**	**	**		*	*	**	**	**	*
Gobio gobio	Gudgeon	F	*						*				*	*							*	
Gobius cobitis	Giant goby	MS			*											*		*				
Gobius couchi	Couch's goby	MM			*																	
Gobius niger	Black goby	MS			*			*							**			**	*			**
Gobius paganellus	Rock goby	ES			*	*		*								*						*
Gobiusculus flavescens	Twospotted goby	MS			**			*				*				*		*	*			*
Gymnammodytes semisquamatus	Smooth sandeel	MS							*													
Gymnocephalus cernuus	Ruffe	FS											*									
Hippocampus guttulatus	Longsnouted seahorse	ES, MS			*														*			
Hippocampus hippocampus	Shortsnouted seahorse	ES, MS																				*
Hyperoplus immaculatus	Corbin's sandeel	ES, MS				*	*		*	**		*	*									
Hyperoplus lanceolatus	Great sandeel	MS	*		*	**	*			*	*	**					*		*	*		
Labrus bergylta	Ballan wrasse	MS			*	*		**			*	*				*		*	*			*
Labrus mixtus	Cuckoo wrasse	MS						*								*						
Lampetra fluviatilis	European river lamprey	A								*			*									
Lepidorhombus whiffiagonis	Megrim	MS												*							*	
Leucaspius delineatus	Sunbleak	FS																	*			
Leuciscus cephalus	Chub	F	*						*				*									
Leuciscus leuciscus	Common dace	F	*						*		**		*	**					*	*		
Limanda limanda	Dab	MM			*		*		*				*					*				
Liparis liparis	Common seasnail	ES, MM						*					*					*			*	
Lipophrys pholis	Shanny	MS			*	*		*				*				*	*		*			**

Liza aurata	Golden grey mullet	MM	**	*	*	*	*	**	*		**	**	*			**	*	*	**		**	**
Liza ramada	Thin lipped grey mullet	C, MM	**	**	*	*	**	**	*	*	**	*		*	**	**	*	*	**	*	**	**
Lumpenus lampretaeformis	Snake blenny	MS																*				
Merlangius merlangus	Whiting	MM, MS				*	*		**	*	*	*	*					*		**	**	*
Microstomus kitt	Lemon sole	MS									*		*									
Mullus surmuletus	Red mullet	MM, MS			*	*		*								*						
Myoxocephalus scorpius	Bullrout	ES, MS		*	*		*	*										*		*		*
Nerophis lumbriciformis	Worm pipefish	ES			*			*		*	*											
Nerophis ophidion	Straightnosed pipefish	ES, MS													*				*			
Oncorhynchus mykiss	Rainbow trout	F																				
Osmerus eperlanus	European smelt	SA		**			*		**	*		*	**		**			*		*		
Parablennius gattorugine	Tompot blenny	MS			*		*	*			*								*			
Pegusa lascaris	Sand sole	MM												*					*	*		
Perca fluviatilis	European perch	F	*						*		*		*							*		
Petromyzon marinus	Sea lamprey	A							*													
Pholis gunnellus	Butterfish	ES, MS		*	*		*	*	*		*					*		*				
Phoxinus phoxinus	Eurasian minnow	F					*	*					*	**					**	*		
Platichthys flesus	Flounder	MM	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**
Pleuronectes platessa	European plaice	MM	*	**	*	*	**	*	**	**	**	**	**	**	*	**	**	**	**	**	**	*
Pollachius pollachius	Pollack	MM, MS		*	*	*	*	**		*	*	*	*	*		*	*		*			
Pollachius virens	Coley	MS								*			*				*					
Pomatoschistus lozanoi	Lozano's goby	MM, MS					*															*
Pomatoschistus microps	Common goby	ES	**	**	**	**	**	**	**	**	**	**	*	**	**	**	**	**	**	**	**	**
Pomatoschistus minutus	Sand goby	ES, MM	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**
Pomatoschistus pictus	Painted goby	MS			**	*	*	*				*			*	*	**		*			*
Psetta maxima	Turbot	MM, MS				*			*			*	*						*			
Pungitius pungitius	Ninespine stickleback	F	*										*									
Raja clavata	Thornback ray	MS			*													*				
Rutilus rutilus	Roach	F	*						*		*		**	*		*			*	*	*	
Salmo salar	Atlantic salmon	A				*		*	*	*			*	**		*	*			*	**	
Salmo trutta	Sea trout	A,F	*		*	*	**	*	*	**	*	*	*	**		*	*			*		*
Sander lucioperca	Zander	FS																				
Sardina pilchardus	European pilchard	MM, MS			*	**		*	*		*											
Scardinius erythrophthalmus	Rudd varieties	F	*					*	*				*			*			*			

Scomber scombrus	Atlantic mackerel	MS			*	*		*														
Scophthalmus rhombus	Brill	MM, MS	*		*	*		*	*		*	*						*	*	*		
Scyliorhinus canicula	Dogfish	MS			*																	
Solea solea	Dover sole	MM	*	*	*	*		*	*		*	*	**	*	*		*	*	*	**	**	*
Sparus aurata	Gilthead bream	MM, MS			*	*													*			*
Spinachia spinachia	Sea stickleback	ES, MS			**		**	*	*			*				*	*		*			*
Spondyliosoma cantharus	Black seabream	MM, MS	*																*			*
Sprattus sprattus	European sprat	MM	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	*
Syngnathus acus	Greater pipefish	ES, MM, MS		*	*	*	*	*	*		*	*	*		*	*		**	*	*		*
Syngnathus rostellatus	Nilsson's pipefish	ES	*	*	*	*	*	*	**		*	*	*	*	*		*	*	*	*		*
Syngnathus typhle	Deepsnouted pipefish	ES, MS			*														*			*
Taurulus bubalis	Longspined sea scorpion	MS		*	*	*	*	*	*		*	*				*		*	*			*
Thymallus thymallus	Grayling	F																	*			
Trachinus draco	Greater weaver	MS							*													
Trachurus trachurus	Atlantic horse mackerel	MS			*	*										*						
Trisopterus esmarkii	Norway pout	MS			*																	
Trisopterus luscus	Pouting	MM						*	*		*											*
Trisopterus minutus	Poor cod	MS						*														
Zoarces viviparus	Viviparous blenny	ES, MS								**		*	*					**				
		Species count	31	25	55	45	37	55	52	22	42	36	48	25	23	34	25	38	49	34	27	41
		Species count in ≤90% of samples per estuary	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	12	12	11	16	11