Processes underpinning fish species composition patterns in estuarine ecosystems worldwide

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ABSTRACT

Aim We present the first global biogeographical regionalization of estuaries, assessing how dispersal limitation and/or environmental filtering mechanisms drive the patterns of fish assemblage composition among and within biogeographical regions.

Location Estuaries worldwide.

Methods A bootstrapped hierarchical cluster analysis was applied to define biogeographical regions based on pairwise beta diversity (βsim) of fish assemblages among 393 estuaries worldwide. Variables representing dispersal limitation and environmental features were used to disentangle the possible effects of assembly processes acting among and within biogeographical regions, through hierarchical partitioning of variation. Finally, we assessed the contribution of species to beta diversity through a method newly developed in this article.

Results Estuaries were grouped into seven major biogeographical regions explained by geographical dispersal variables. Dissimilarity in species composition within each biogeographical region was mainly determined by ecosystem-connectivity dispersal variables, with a minor influence of environmental variables (chiefly sea surface temperature). Marine species contributed highly to beta diversity between estuaries but less than expected by chance, whilst freshwater species had low contributions yet also less than expected. Estuarine (i.e. brackish) and diadromous species had low contributions to beta diversity but higher than expected by chance.

Main conclusions Fish assemblage composition in estuaries seems to be driven by dispersal limitation processes both among and within biogeographical regions, with only a minor role of environmental filtering evident within biogeographical regions. These findings contrast with the acknowledged importance of environmental variables as drivers of species richness patterns. Irrespective of biogeographical region, the ecosystem affinity of a species determined its contribution to beta diversity between estuaries. The new method presented here offers new opportunities for assessing the effects of taxonomic and functional aspects on beta diversity (βsim) across taxa and ecosystems, and thus contributes to improving knowledge of assembly processes.

Keywords assembly mechanisms, beta diversity, biodiversity, biogeography, coastal, community, functional, global scale, regional scale, taxonomic

INTRODUCTION

A thorough understanding of biodiversity patterns and associated driving mechanisms is key to test hypotheses about spatial organization and to improve our knowledge of large scale functioning of biological assemblages (Bellwood & Hughes, 2001; Soininen et al., 2007; Bender et al., 2013). Global patterns of fish assemblages have been
characterized in both marine (e.g. Bellwood & Wainwright, 2002; Spalding et al., 2007; Floeter et al., 2008; Tittensor et al., 2010; Briggs & Bowen, 2012; Bender et al., 2013; Cowman et al., 2013; Kulbicki et al., 2013) and freshwater ecosystems (e.g. Abell et al., 2008; Leprieur et al., 2011; Villéger et al., 2011) but remain poorly understood in estuaries and coastal lagoons despite recent advances in our understanding of global patterns of species richness (Vasconcelos et al., 2015), and numerous ecological studies at local and regional scales (e.g. Harrison & Whitfield, 2006; Zapata & Robertson, 2007; Sheaves & Johnston, 2009; Nicolas et al., 2010; Francis et al., 2011; Sheaves, 2012).

Beta diversity, defined as compositional variation across space or time, has been widely used to analyse spatial patterns of assemblages and respective changes at different spatial scales (Soininen et al., 2007; Graham & Fine, 2008; Barton et al., 2013). Its use in conservation planning is motivated by the diversity-stability hypothesis, which advocates that higher beta diversity within communities increases resistance to disturbances and thus buffers the risk of ecological collapse - even if the theoretical expectation of negative relationship between spatial and temporal turnover was only recently empirically demonstrated at community level (Melin et al., 2014).

Ecological theory suggests that compositional differences among biological assemblages are determined by dispersal limitation, environmental filtering and biotic interactions (Legendre et al., 2005; Rice, 2005; Graham & Fine, 2008; Gotzenberger et al., 2012; Barton et al., 2013). Dispersal limitation is related to speciation-extinction events (i.e. evolutionary processes) associated with the existence of historical and geographical barriers (e.g. plate tectonic events) that promote isolation and consequentially spatial dissimilarity between biological assemblages (Legendre et al., 2005; Barton et al., 2013). Environmental filtering occurs when environmental constraints give rise to spatial niche segregation among species (i.e. niche-based assembly rules), favouring speciation and species turnover (Legendre et al., 2005; Barton et al., 2013). Finally, biotic interactions (e.g. competition, tolerance, adaptation, recruitment) generate spatial autocorrelation in the coexistence of species and a gradient in species composition, with assemblages dominated by species with a competitive advantage (Legendre et al., 2005; Rice, 2005).

These assembly mechanisms can act simultaneously and the importance of each one is linked with the spatial and temporal scale of analysis (Fig. 1; Hillebrand & Blenchner, 2002; Graham & Fine, 2008; Leprieur et al., 2009; Gotzenberger et al., 2012; Barton et al., 2013; De Juan et al., 2013). At a global scale (Fig. 1A) it has been suggested that dispersal limitation determines the species pool over geological time, as biogeographical barriers limit connectivity, promote the isolation of populations and assemblages and lead to speciation and radiation events (Hillebrand & Blenchner, 2002; Gotzenberger et al., 2012; Barton et al., 2013). At regional scales (Fig. 1B), species are also limited by dispersal ability, as well as by habitat suitability and physiological tolerance, and thus at this scale both dispersal limitation and environmental filtering mechanisms play an important role in shaping species assemblages (Hillebrand & Blenchner, 2002; Gotzenberger et al., 2012; Barton et al., 2013). At this spatial scale, dispersal limitation is mediated by habitat patchiness and connectivity (e.g. topology, discontinuous habitat, dispersal corridors) (Hillebrand & Blenchner, 2002; Leprieur et al., 2009; Barton et al., 2013).

Finally, at local scales (e.g. within and between habitat patches) (Fig. 1C), local dispersal limitation supposedly diminishes, and assemblage composition results from limitations imposed by environmental filtering and biotic interactions, that act upon the regional species pool depending on the niche requirements of species (Hillebrand & Blenchner, 2002; Floeter et al., 2008; Gotzenberger et al., 2012; Barton et al., 2013).

Patterns of beta diversity along large-scale gradients differ among ecosystems and groups of organisms (Soininen et al., 2007; Qian & Ricklefs, 2012; Barton et al., 2013). In general, beta diversity increases with habitat heterogeneity and fragmentation, and therefore lower beta diversity is expected in marine ecosystems than in terrestrial and freshwater ecosystems (river basins) (Soininen et al., 2007). Furthermore, greater homogeneity in composition between biological assemblages is expected as the dispersal ability of species increases (Soininen et al., 2007). Since estuaries and coastal lagoons are transitional ecosystems, their assemblages comprise different proportions of fish species from both adjacent marine and freshwater environments that are able to tolerate the transitional conditions, as well as estuarine and migratory diadromous species (Whitfield et al., 2012). It is therefore reasonable to assume that estuaries, as transitional ecosystems, are more fragmented than marine ecosystems but less than freshwater ecosystems. An integrative approach on assemblage patterns at large spatial scales should elucidate on processes generating patterns of beta diversity among estuarine ecosystems.

Using a comprehensive worldwide database of fish assemblages in estuaries (393 estuaries and 2514 species) we investigate global estuarine biogeography for the first time, and determine how dispersal limitation and/or environmental filtering mechanisms drive patterns of fish assemblage composition among and within the biogeographical regions defined. We address three independent hypotheses for estuarine ecosystems:

1. At a global scale (i.e. among biogeographical regions), differences in fish assemblage composition among estuaries are driven by dispersal limitation processes rather than environmental filtering (see Fig. 1A). Therefore, global biogeographical patterns observed are the result of isolation processes due to species dispersal limitations, and reflect known geographical barriers in freshwater and marine ecosystems (i.e. geographical dispersal variables) (see Fig. 1A).
Both dispersal limitation and environmental filtering drive beta diversity between estuaries at a regional scale (i.e. within biogeographical regions). At this scale, differences in composition of fish assemblages between estuaries are related to ecosystem patchiness and connectivity (i.e. ecosystem-connectivity dispersal variables) as well as with environmental variables (see Fig. 1B).

3. Fish species with different affinities (i.e. marine, freshwater, estuarine and diadromous, see Fig. 1) contribute differently to the observed beta diversity between estuaries, as a result of their different proportion within assemblages and their intrinsic dispersal capacity (i.e. beta diversity decreases with species dispersal). Species from more fragmented environments (i.e. freshwater, estuarine) may have a disproportionately high contribution to beta diversity even if they sum to a low proportion of the assemblage. Specifically, to test this hypothesis, we develop and present a new method to quantify the contribution of each species to beta diversity.

## MATERIALS AND METHODS

### Fish assemblage database

The present study relied on a database that included 393 estuaries and coastal lagoons distributed worldwide (see Appendix S1 in Supporting Information). The database was compiled from published literature and each sample in the database ($n = 567$ samples) corresponded to the total fish species list of the sampled assemblage in an individual estuary in a given study (presence/absence data) (see Appendix S2). To minimize the bias of different sampling methods, the database only included studies that used active fishing gear, namely trawl, seine, beach seine, purse seine, cast net and/or hand/dip net. For each study, the total area sampled in an estuary was estimated based on the area covered by each replicate, the number of sites sampled and the temporal frequency of sampling. All studies were kept in the database since no significant relationship was found between pairwise dissimilarity in terms of species composition (Simpson dissimilarity) and total area sampled (Euclidean distances) \[\text{Mantel test } r = 0.021, P > 0.05; \text{Pearson's product-moment correlation method using 4999 permutations; package 'vegan' in R (Oksanen et al., 2015).}\]

Each species in the database (2514 species) was characterized in terms of ecosystem affinity [marine, freshwater, estuarine (i.e. brackish), diadromous] with information extracted from FishBase database (Froese & Pauly, 2014) and complemented with available literature.

### Geographical dispersal, ecosystem-connectivity dispersal and environmental variables

For each estuary we determined a set of variables based on published literature, databases and geospatial vector data (see details in Appendix S2). These variables were grouped according to their relation to assembly mechanisms (see Fig. 1).

1. Geographical dispersal variables considered were continental and marine biogeographical realm (see Fig. 2, hereafter realm). The straight line Euclidean distance was measured between mid-points of realms and continents (km). These variables were considered categorical proxies of the evolutionary history of biogeographical regions, and measured the effects of dispersal limitation responsible for the isolation of biological assemblages at a global scale.

2. Ecosystem-connectivity dispersal variables comprised those related with the connectivity of estuaries and these with the adjacent marine environment, specifically tidal range (macro-, meso-, microtidal), estuary mouth width (m), estuary type (open/temporarily open) and geographical distance as a proxy for environmental dissimilarity (straight line Euclidean distance between estuaries, measured at the mouth). These variables measured dispersal limitation effects operating at regional scales.

3. Several environmental variables were used to assess environmental filtering effects. Since beta diversity is expected to
increase with habitat heterogeneity, which is itself expected to increase with habitat area (Heino et al., 2015), the variables estuary area (km²), drainage basin area (km²) and continental shelf width (km) were included to represent availability of estuarine, freshwater and marine habitats, respectively. Temperature (mean sea surface temperature in °C at the estuary mouth) and estuary salinity type (regular, regular to hyperhaline, hyperhaline) were also included and addressed species physiological tolerance to habitat conditions, namely temperature and salinity.

**Statistical analyses**

All analyses were based on pairwise dissimilarities between assemblages, quantified from species presence–absence data. Simpson dissimilarity ($\beta_{\text{sim}}$) was chosen to measure assemblage dissimilarity since it is not affected by variation in species richness (nestedness) and quantifies only the turnover or replacement of species (Koleff et al., 2003). Assemblage turnover, as quantified by $\beta_{\text{sim}}$, allows us to describe assemblage patterns (delineating biogeographical regions), which was the goal of this analysis, rather than characterize biological processes that drive the observed patterns namely species replacement, which would require an index such as $\beta_3$ (Carvalho et al., 2012). A hierarchical cluster analysis using a reclustering procedure was implemented to deal with any bias resulting from sample order in the original presence–absence matrix as adjacent pairs of sites are more likely to be linked during the regular clustering procedure (Dapporto et al., 2013). This method creates a final consensus tree after reshuffling sample
order (which can produce different output trees at each agglomeration step) and assesses the robustness of cluster nodes in the tree through bootstrapping (Dapporto et al., 2013). The βsim dissimilarity matrix was calculated and cluster analysis performed using package ‘recluster’ (Dapporto et al., 2013) in R. Ward’s minimum variance method was used as the agglomerative clustering algorithm. This method reduces the number of clusters with a single sample, minimizing total within-cluster variation, and is suitable for large-scale studies (Kulbicki et al., 2013). A bootstrap consensus tree was produced from 1000 trees – each of these trees obtained after randomly shuffling the matrix row order 100 times (and considering a 50% majority rule consensus tree), and with a bootstrap sampling of 100 species. Subsequently, the relationship among clusters in the final tree was analysed based on the mean dissimilarities between estuaries in each pair of clusters.

Mantel tests were used to quantify the relationship between pairwise dissimilarity among assemblages (βsim) and dispersal and environmental variables (Euclidean distance). Tests were conducted using Spearman rank correlation coefficient to search for nonlinear and linear relationships (significance measured with 9999 permutations). Additionally, the relative importance of each variable to βsim was assessed through hierarchical partitioning of variation, which determines the independent contribution of each variable by measuring the difference in $R^2$ of models with and without the variable, while avoiding collinearity problems (Carvalho & Cardoso, 2014). Mantel tests and hierarchical partitioning of variation were performed among and within the biogeographical regions identified with the cluster analysis. To test hypotheses 1 and 2, several models were constructed with variation in βsim explained by: (1) dispersal limitation and environmental filtering processes (geographical dispersal, ecosystem-connectivity dispersal and environmental variables); (2) dispersal limitation processes (i.e. geographical dispersal and ecosystem-connectivity dispersal variables); (3) environmental filtering processes (i.e. environmental variables). An additional model (4) with geographical distance and environmental variables assessed if geographical distance acted as a proxy of environmental distance. Analyses were implemented using the packages ‘vegan’ (Oksanen et al., 2015) and ‘relaimpo’ (Groemping, 2006) in R.

To test hypothesis 3, a method was developed to measure the contribution of individual species to $\beta_{\text{sim}}$. The method relies on two facts: (i) Given two communities (comm$_1$ and comm$_2$), $\beta_{\text{sim}}$ quantifies the turnover between them and each community contributes equally to it:

$$C_{\text{comm}} = \frac{\beta_{\text{sim}}}{2}; \quad C_{\text{comm}} = \frac{\beta_{\text{sim}}}{2}$$

where $C_{\text{comm}}$ is the partial contribution of each community to $\beta_{\text{sim}}$. (ii) As species are considered ecologically equivalent in taxon diversity measures (Cardoso et al., 2014), each species contributes equally to the partial contribution of each community to $\beta_{\text{sim}}$:

$$C_{\text{sp}} = \frac{C_{\text{comm}}}{U_{\text{comm}}}; \quad C_{\text{sp}} = \frac{C_{\text{comm}}}{U_{\text{comm}}}$$

where $C_{\text{sp}}$ is the contribution of each species unique to a given community in the pairwise comparison and $U_{\text{comm}}$ is the number of species unique to that community. This formulation also guarantees the intuitive property expressed as:

$$\beta_{\text{sim}} = \sum C_{\text{sp}}$$

Using this method, the contribution of each fish species to the dissimilarity between assemblages in estuaries was measured as its mean contribution to $\beta_{\text{sim}}$ among all pairwise comparisons (including sites where it does not occur). The overall contribution of each group of species (marine, freshwater, estuarine, diadromous) was measured by summing the individual contributions of their species. Finally, to test if the contribution of each group to $\beta_{\text{sim}}$ observed between assemblages (among and within estuarine biogeographical regions) was different than could be expected by chance, we compared the observed value with 999 permutations of species between groups, keeping the species richness per group constant. This allowed us to determine if the contribution of some groups to beta diversity was disproportionally larger or smaller than expected if species contributions were randomly assigned to groups.

**RESULTS**

Seven groups of estuaries were obtained with cluster nodes recovered in the final bootstrapped consensus tree (clusters A-G; Fig. 2; henceforward designated as estuarine biogeographical regions). Additional sub-divisions of these clusters were not supported (with the next level of nodes recovered in 45–100% of bootstrap replicates in clusters B, D, F, G, and in 0–1% of bootstrap replicates in clusters A, C, E).

Cluster A (Indo-Pacific) comprised all analysed estuaries in Indo-Pacific and Tropical Atlantic realms (East) (Fig. 2). Cluster B (Tropical Eastern Pacific) included all estuaries in the Tropical Eastern Pacific realm. Estuaries in the Temperate Northern Pacific realm (East) were grouped with cold-temperate estuaries of Temperate Northern Atlantic realm (West) (Cluster C – Cold-temperate North America). All other estuaries on the eastern American coast south of Long Island Sound (including the Gulf of Mexico) were clustered regardless of their realm (Cluster E – Warm and Warm-temperate Western Atlantic). Cluster D (Temperate Australasia) included estuaries in the Temperate Australasia realm, while cluster F (Eastern Temperate North Atlantic) included estuaries from the Temperate Northern Atlantic (East), and cluster G (Temperate Southern Africa) estuaries from the Temperate Southern Africa realm. Mean beta diversity of clusters revealed a spatial pattern (Fig. 3), with three major groups identified (i.e. Clusters E and B, in America; Clusters F and C, in North America and Europe; Clusters G, D and A across Africa, Asia and Oceania).
Hierarchical partitioning of variation (Table 1) showed that most beta diversity among biogeographical regions was explained by geographical dispersal variables and geographical distance, suggesting a strong influence of dispersal limitation processes at this global scale. Moreover, these variables and those relating to ecosystem-connectivity dispersal (namely characterizing the connectivity of estuaries with the marine ecosystem: estuary mouth width, estuary type, tidal range) explained a high proportion of assemblage dissimilarity within biogeographical regions, showing the importance of dispersal limitation processes at this scale. Sea surface temperature was the only environmental variable relevant at both spatial scales, whilst estuary area and continental shelf width explained a small proportion of variation within biogeographical regions B and D. Overall, considerably higher proportions of variance were explained entirely by dispersal limitation processes than by environmental filtering processes, both among and within estuarine biogeographical regions. The exceptions are clusters F and G where both processes are likely influential, since sea surface temperature was important and geographical dispersal variables were not important (each of these clusters included estuaries from a single continent and marine biogeographic realm).

When hierarchical partitioning of variation included environmental variables and geographical distance (see Appendix S3), the latter explained a high proportion of variation (35% in global model) and environmental variables maintained the proportions obtained in previous models with environmental variables only (Table 1; change < 5% in global model). Also, correlations between geographical distance and environmental variables were very low \((r < 0.03)\) Mantel tests, \(P < 0.05;\) results not shown) thus geographical distance was not a proxy for environmental distance.

Mantel tests (Table 2) revealed a strong and consistent relation of beta diversity (among and within biogeographical regions) with ecosystem-connectivity dispersal variables, mainly geographical distance and, to a lesser degree, estuary mouth width and tidal range. Beta-diversity was also consistently related with some environmental variables, namely sea surface temperature and to a lesser degree continental shelf width and estuary area.

Higher species richness (Table 3) and mean within-cluster beta diversity (Fig. 4) were observed for clusters A and E – which cover wide spatial extents – and high mean within-cluster beta diversity was also found for cluster C. Conversely, lower species richness and mean within-cluster beta diversity were found for cluster G that is restricted to Temperate Southern Africa. All estuarine biogeographical regions had a large proportion of exclusive species (i.e. not shared with other clusters) (Table 3). In terms of assemblage composition (Table 3), estuaries were dominated by marine fish species, which also had the highest contribution to beta diversity. Estuarine and freshwater species were also relevant in terms of species richness globally, particularly in clusters A, E and G. Nevertheless, species with different ecosystem affinities contributed differently to beta diversity (Table 3): marine species contributed highly to \(\beta_{\text{sim}}\) but less than expected by chance (considering their high species richness); the contributions of estuarine and diadromous species to \(\beta_{\text{sim}}\) were low but higher than expected by chance (considering their low species richness); and the contributions of freshwater species to \(\beta_{\text{sim}}\) were also low but less than expected by chance.

**DISCUSSION**

**Estuarine biogeography and assembly mechanisms among biogeographical regions**

This study is the first to address the global biogeography of fish assemblages of estuaries as well as the associated driving assembly mechanisms. Cluster analysis of \(\beta_{\text{sim}}\) showed a clear division of fish assemblages into seven major estuarine biogeographical regions, which are in general agreement with previously described global biogeographical patterns of fish assemblages in freshwater and marine ecosystems (e.g. Bellwood & Hughes, 2001; Spalding et al., 2007; Abell et al., 2008; Floeter et al., 2008; Tittensor et al., 2010; Leprieur et al., 2011; Villéger et al., 2011; Briggs & Bowen, 2012; Bender et al., 2013; Cowman et al., 2013; Kulbicki et al., 2013). The observed pattern had greater affinity with marine than with freshwater biogeographical regions, which concurs with the dominance of marine fish species within estuaries (Elliott et al., 2007).

The processes responsible for the observed global patterns of fish assemblages in estuaries were clarified by hierarchical partitioning of variation, with geographical dispersal variables explaining an important proportion of variation, followed by the ecosystem-connectivity dispersal variable geographical distance. This supports the hypothesis that the global pattern of beta diversity of assemblages (i.e. among estuarine biogeographical regions) is mainly linked to
Table 1 Hierarchical partitioning models fitted to beta diversity among assemblages in estuaries and explanatory variables related with assembly mechanisms (All – dispersal limitation and environmental filtering processes, Disp – dispersal limitation process, Env – environmental filtering process). Two scales were addressed: global and regional, i.e. respectively, among and within estuarine biogeographical regions shown in Fig. 2 (A – Indo-Pacific, B – Tropical Eastern Pacific, C – Cold-temperate North America, D – Temperate Australasia, E – Warm and Warm-temperate Western Atlantic, F – Eastern Temperate North Atlantic, G – Temperate Southern Africa). Values are the proportion of variance explained by each variable and by the model.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Global</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
</tr>
</thead>
<tbody>
<tr>
<td>Geographical dispersal</td>
<td>Marine biogeographical realm</td>
<td>All</td>
<td>Disp</td>
<td>Env</td>
<td>All</td>
<td>Disp</td>
<td>Env</td>
<td>All</td>
</tr>
<tr>
<td>Continent</td>
<td>11.04</td>
<td>11.71</td>
<td>12.70</td>
<td>14.80</td>
<td>1.80</td>
<td>1.70</td>
<td>24.50</td>
<td>29.00</td>
</tr>
<tr>
<td>Geographical distance</td>
<td>12.49</td>
<td>12.55</td>
<td>9.50</td>
<td>9.39</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>na</td>
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<tr>
<td>Ecosystem-connectivity dispersal</td>
<td>Estuary mouth width</td>
<td>14.57</td>
<td>15.95</td>
<td>16.60</td>
<td>16.70</td>
<td>16.10</td>
<td>17.80</td>
<td>34.10</td>
</tr>
<tr>
<td>Estuary type</td>
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<td>0.54</td>
<td>3.60</td>
<td>5.20</td>
<td>3.80</td>
<td>4.90</td>
<td>1.10</td>
<td>1.80</td>
</tr>
<tr>
<td>Tidal range</td>
<td>3.76</td>
<td>5.67</td>
<td>2.10</td>
<td>2.50</td>
<td>20.80</td>
<td>21.30</td>
<td>1.30</td>
<td>1.60</td>
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<td>7.76</td>
<td>14.39</td>
<td>10.60</td>
<td>17.17</td>
<td>2.68</td>
<td>3.00</td>
<td>14.50</td>
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<td>Continental shelf width</td>
<td>0.76</td>
<td>0.90</td>
<td>0.89</td>
<td>1.44</td>
<td>1.83</td>
<td>3.48</td>
<td>1.08</td>
<td>3.14</td>
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<tr>
<td>Estuary area</td>
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<td>0.31</td>
<td>1.70</td>
<td>1.59</td>
<td>3.75</td>
<td>14.56</td>
<td>0.40</td>
<td>0.51</td>
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<tr>
<td>Basin area</td>
<td>0.11</td>
<td>0.19</td>
<td>1.70</td>
<td>1.56</td>
<td>0.20</td>
<td>0.72</td>
<td>0.05</td>
<td>0.04</td>
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<td>0.10</td>
<td>0.07</td>
<td>0.27</td>
<td>0.12</td>
<td>0.30</td>
<td>0.77</td>
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<tr>
<td>% of variation explained by model</td>
<td>51.3</td>
<td>46.6</td>
<td>16.4</td>
<td>60.6</td>
<td>50.0</td>
<td>21.8</td>
<td>63.1</td>
<td>59.0</td>
</tr>
</tbody>
</table>
dispersal limitation processes (Barton et al., 2013). Notwithstanding, the role of sea surface temperature identified suggests that environmental filtering processes also shape assemblage composition at this global scale. Temperature creates suitable or unsuitable environmental conditions — dependent on the physiological tolerance of each species and independent of its dispersal capability — determining success-ful establishment and consequently species range limits at large spatial scales (Wiens, 2011). And as a result species in estuaries are distributed along a global temperature gradient as in other aquatic ecosystems (e.g. Mellin et al., 2016). For instance, Briggs & Bowen (2012) proposed a realignment of marine biogeographical provinces with the merger of warm-temperate and tropical regions as well as of cold and cold-temperate regions, resulting in longitudinal dispersal gradients.

Similarity in fish assemblage composition among estuarine biogeographical regions (i.e. three groups of clusters, see Fig. 3) was consistent with a vicariance scenario (associated with the appearance of geographical barriers) as well as temperature filters (Fig. 2). The affinity between estuaries of clusters B and E (Tropical Eastern Pacific, Warm and Warm-temperate Western Atlantic) is consistent with the connection between these faunas until the recent closure of the Isthmus of Panama 3.1–3.5 Ma (hard barrier, Fig. 2). In addition, these two regions are isolated from others to the North (cluster C – Cold-temperate North America) by temperature filters that constrain the establishment of tropical species in cold-temperate regions (Zapata & Robertson, 2007; Briggs & Bowen, 2012) and by hard barriers that restrict longitudinal dispersal of shallow-water species (Bellwood & Wainwright, 2002; Spalding et al., 2007; Zapata & Robertson, 2007; Floeter et al., 2008; Kulbicki et al., 2013), such as the East Pacific Barrier (5000 km wide deep open ocean area – bathymetric barrier with the Indo-Pacific) west of cluster B, and the Mid-Atlantic Ridge (bathymetric barrier between the Western and Eastern Atlantic) east of Cluster E (Fig. 2). This pattern highlights the importance of dispersal limitation and temperature filters in determining species range limits by showing that even areas across oceans have relatively similar fish assemblages if they are linked by their evolutionary history (Briggs, 2007; Briggs & Bowen, 2012).

Temperature filters are responsible for the southern delimitation of cluster F (Eastern temperate North Atlantic), while hard barriers limit the longitudinal dispersal of species to its west (Mid Atlantic Ridge and Red Sea land bridge; Fig. 2) as well as west of cluster C (Cold-temperate North America; East Pacific Barrier and former Bering land bridge; Fig. 2) (Bellwood & Wainwright, 2002; Briggs, 2007; Spalding et al., 2007; Floeter et al., 2008; Briggs & Bowen, 2012; Cowman et al., 2013). Cluster E closely matches the biogeographical Neotropical region and its freshwater fishes (Leprieur et al., 2011; Villéger et al., 2011), which is in agreement with the notably high proportion of freshwater species in this cluster (24%).

The lack of hard barriers within the Indo-Pacific (isolated from the Atlantic due to the Red Sea Land Barrier since the Miocene), has enabled many species to maintain widespread geographical distributions (Cowman et al., 2013), and explains the similarity among clusters A, D and G (Indo-Pacific, Temperate Australasia and Temperate Southern Africa, respectively). Nonetheless, soft barriers to fish species distributions (e.g. temperature and currents) have resulted in three different estuarine biogeographical regions. The Temperate Southern Africa region is defined by temperature filters (Fig. 2), and species dispersal among its estuaries may be facilitated by currents such as the Indian – Atlantic surface flow and the Benguela and Agulhas local currents (Harrison & Whitfield, 2006; Kuhlbrodt et al., 2007). Fish assemblages from estuaries in the Indo-Pacific and Temperate Australasia estuarine biogeographical regions were separated as in previous studies of marine fish (e.g. Bellwood &

<table>
<thead>
<tr>
<th>Clusters</th>
<th>Geographical dispersal</th>
<th>Ecosystem-connectivity dispersal</th>
<th>Environmental</th>
<th>Salinity type</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Geographical realm</td>
<td>Estuary type</td>
<td>Sea surface temperature</td>
<td>USGS 2008-2011</td>
</tr>
<tr>
<td></td>
<td>Marine biogeographical</td>
<td>Tidal range</td>
<td>Continental shelf width</td>
<td>usgs 2008-2011</td>
</tr>
<tr>
<td></td>
<td>realm</td>
<td>Estuary area</td>
<td>Estuary area</td>
<td>usgs 2008-2011</td>
</tr>
<tr>
<td></td>
<td>Continent</td>
<td>Basin area</td>
<td>Basin area</td>
<td>usgs 2008-2011</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Salinity type</td>
<td>Salinity type</td>
<td>usgs 2008-2011</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ns</td>
<td>ns</td>
<td>usgs 2008-2011</td>
</tr>
</tbody>
</table>

### Table 2

Results of Mantel tests using Spearman rank correlation between beta diversity of fish assemblages and each explanatory variable, at two spatial scales: global and regional (i.e. respectively, among and within estuarine biogeographical regions shown in Fig. 2 (A – Indo-Pacific, B – Tropical Eastern Pacific, C – Cold-temperate North America, D – Temperate Australasia, E – Warm and Warm-temperate Western Atlantic, F – Eastern Temperate North Atlantic, G – Temperate Southern Africa). Values shown are r_M statistic.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Global</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
</tr>
</thead>
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<tr>
<td>Geographical dispersal</td>
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<td>0.439***</td>
<td>0.752***</td>
<td>ns</td>
<td>0.817***</td>
<td>–</td>
<td>0.649***</td>
<td>–</td>
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<tr>
<td></td>
<td>Continent</td>
<td>0.409***</td>
<td>0.424***</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0.693***</td>
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<tr>
<td>Ecosystem-connectivity dispersal</td>
<td>Geographical distance</td>
<td>0.497***</td>
<td>0.672***</td>
<td>0.682***</td>
<td>0.843***</td>
<td>0.664***</td>
<td>0.729***</td>
<td>0.608***</td>
</tr>
<tr>
<td></td>
<td>Estuary mouth width</td>
<td>0.193***</td>
<td>0.563***</td>
<td>0.600***</td>
<td>0.224***</td>
<td>0.361***</td>
<td>0.157***</td>
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<td></td>
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<td>ns</td>
<td>0.496***</td>
<td>ns</td>
<td>ns</td>
<td>0.253***</td>
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<td>Tidal range</td>
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<td>0.282***</td>
<td>0.735***</td>
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<td>0.307***</td>
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<td>0.439***</td>
<td>0.377***</td>
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<td>Continental shelf width</td>
<td>0.203***</td>
<td>0.253**</td>
<td>0.197*</td>
<td>0.316***</td>
<td>0.356***</td>
<td>0.128**</td>
<td>0.274**</td>
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<tr>
<td></td>
<td>Estuary area</td>
<td>0.191***</td>
<td>0.666***</td>
<td>0.366***</td>
<td>0.073*</td>
<td>0.325***</td>
<td>0.148**</td>
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<td></td>
<td>Basin area</td>
<td>0.085***</td>
<td>0.457***</td>
<td>ns</td>
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<td>0.198***</td>
<td>0.187***</td>
<td>ns</td>
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<td>Salinity type</td>
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<td>ns</td>
<td>ns</td>
<td>0.089**</td>
<td>0.267***</td>
<td>0.608***</td>
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</tbody>
</table>

***P < 0.001, **P < 0.01, *P < 0.05, ns P > 0.05.
The Central Indo-Pacific has been considered a macroevolutionary source of biodiversity due to its extensive shallow-water geological complexity, with few geographical barriers, and its position between two major biogeographical regions in Indian and Pacific oceans (Bellwood & Hughes, 2001; Bellwood & Wainwright, 2002; Tittensor et al., 2010; Bender et al., 2013; Cowman et al., 2013; Mouillot et al., 2013). These features promote species survival, dispersal and range expansion as well as connectivity among habitats, favouring diversification (Bellwood & Hughes, 2001; Bellwood & Wainwright, 2002; Tittensor et al., 2010; Bender et al., 2013; Cowman et al., 2013; Mouillot et al., 2013), and explaining the high biodiversity (i.e. species richness and turnover) and large area of the Indo-Pacific estuarine biogeographical region. The dissimilarity between the former region and Temperate Australasia reflect a history of geological events and local features: the boundary between them coincides with the separation of tropical and warm-temperate waters, and their fish assemblages are influenced by the East Australian (poleward-flowing western) and Leeuwin (poleward-flowing eastern) currents (Butler et al., 2010).

In summary, known geographical barriers that limit species dispersal can be directly linked with the identified estuarine biogeographical regions and support our hypothesis 1 (i.e. dispersal limitation processes drive global diversity patterns of estuaries). However, at this global scale, environmental filtering processes also contribute to define fish assemblages' composition along a large-scale climatic gradient of temperature.
Finally, a considerable proportion of beta diversity patterns was explained by the deterministic processes of dispersal limitation and environmental filtering due to temperature (see Table 1), yet so-called stochastic or neutral processes (i.e. ‘chance’ colonization, ‘random’ extinction and ecological drift) cannot be completely disregarded, as they possibly affect species composition (Vellend, 2010; Chase & Myers, 2011). If species were ecologically equivalent, in the absence of limits to dispersal, species could be distributed everywhere and spatial patterns of biodiversity would be absent or due to chance (Wiens, 2011). Nevertheless, neutrality is expected to have a stronger influence in smaller or more isolated habitats (Chase & Myers, 2011). Therefore, at the spatial scales analysed here (global and regional) it seems unlikely that neutral processes (Wiens, 2011) would have a large influence on species assemblages given the strong relationship between the observed patterns with well-established large-scale climatic gradients as well as marine and freshwater biogeographies. Thus, the unexplained variance could be primarily due to unmeasured explanatory variables, such as human pressure and climate variability that can be strongly associated with species occurrence patterns, as shown for coral reef fish (Mellin et al., 2016).

**Assembly mechanisms within biogeographical regions**

The present results support our hypothesis 2 that dispersal limitation and environmental filtering processes influence fish assemblage composition among estuaries at a regional scale (see Fig. 1). However, dispersal variables (i.e. representing dispersal limitation processes) were more important in explaining beta diversity than environmental variables (i.e. those representing environmental filtering processes), even though sea surface temperature had a larger influence within estuarine biogeographical regions than among regions. Hierarchical partitioning of variation and Mantel tests rendered no consistent pattern of other environmental variables within biogeographical regions, although continental shelf width and estuary area explained some variation in a few models.

Sea surface temperature seems to limit species distributions range (through physiological constraints) at global and also at regional scales. But the other analysed environmental variables were not influential drivers of assemblage composition at this regional scale. However, at smaller spatial scales (i.e. smaller regions or locally), species composition has been for example related to salinity, turbidity and depth in South African estuaries (Harrison & Whitfield, 2006), and to habitat area and sediment type in tropical north Queensland (Australia) (Sheaves & Johnston, 2009).

It seems relevant to compare the drivers of species composition patterns with those of species richness obtained at equivalent spatial scales. Despite the low importance of environmental variables (except sea surface temperature) for species composition patterns within biogeographical regions, these environmental variables strongly regulate species richness (e.g. Harrison & Whitfield, 2006; Nicolas et al., 2010; Vasconcelos et al., 2015). Overall, the results point to an influence of dispersal limitation and environmental filtering mechanisms on species composition between estuaries with the latter increasingly more important at regional than global scales. Further studies should include a larger set of spatial scales (from local, i.e. estuary-level, to regional), replicated worldwide, to test the idea that dispersal limitation processes have a much greater influence than environmental filtering processes on assemblage composition globally and regionally, in contrast with species richness which at these scales is strongly driven by both processes (Vasconcelos et al., 2015).

**Species contribution to beta diversity**

The results clearly support our hypothesis 3 that the contribution of species to beta diversity is related to their ecosystem affinity; with marine and freshwater species having lower than expected contributions to \( \delta \)sim whilst the contributions of estuarine and diadromous species, even if low, were higher than expected considering their comparatively lower species richness. Evidence for a link between species affinities and their contribution to beta diversity via dispersal limitation has, to our knowledge, not been shown before.

Marine and freshwater species represent high proportions of assemblages in estuaries, and are unlikely to co-occur in the same habitat within an estuary due to their divergent salinity tolerances (i.e. marine species typically occur downstream and freshwater species upstream; Fig. 2) (Whitfield et al., 2012). Furthermore, estuarine species as well as diadromous species correspond to small proportions of these assemblages (Whitfield et al., 2012). Therefore, differences in species contribution to the observed patterns of beta diversity are likely to be a consequence of dispersal limitation. The same pattern was found across both spatial scales analysed, further emphasizing the consistency of these findings. High connectivity among marine habitats facilitates species dispersal promoting homogeneity among assemblages and low beta diversity (Soininen et al., 2007), thus explaining why the contribution of marine species to \( \delta \)sim was lower than what could be expected despite their dominance within assemblages. Conversely, the high degree of natural isolation of less connected habitats such as estuaries and river basins would suggest high contributions of estuarine (despite their low proportion) and freshwater species to beta diversity among assemblages of estuaries (Soininen et al., 2007; Leprieur et al., 2011). The results supported this hypothesis for estuarine species, but not for freshwater species despite the low habitat connectivity between freshwater habitats. This might be due to the geographical patterns of fish beta diversity in freshwater ecosystems worldwide that are largely controlled by either climatic stability or severe climatic changes (Leprieur et al., 2011). Due to their migratory behaviour, diadromous species have high mobility and environmental
tolerance. Their pronounced contribution to beta diversity is probably related with their typically rare and markedly seasonal occurrence within estuaries (Whitfield et al., 2012). These findings suggest that mobility and dispersal limitation of species, together with their rarity (e.g. seasonal effects) define their contribution to assemblage dissimilarity.

Ultimately, large spatial scale studies on fish assemblages in estuaries should now explore other facets of biodiversity, beyond taxonomic composition. Namely, investigating phylogenetic and functional diversity should advance our understanding on how shared evolutionary history, resource partitioning and other functional features of assemblages (e.g. body size, trophic level) relate with assembly mechanisms (e.g. Soininen et al., 2007; Bender et al., 2013). The complementarity between taxonomic, phylogenetic and functional approaches should have broad applications in emerging fields of macroecology, and will improve our knowledge on the processes that structure biological assemblages (Villéger et al., 2013; Cardoso et al., 2014).

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REFERENCES


**SUPPORTING INFORMATION**

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Estuaries and references of fish assemblages database.

Appendix S2 Description of the construction of fish assemblages database as well as of dispersal and environmental variables used in the analyses.

Appendix S3 Hierarchical partitioning models fitted to beta-diversity.

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**BIOSKETCH**

**Sofia Henriques** is interested in the integration of taxonomic and functional structure of fish assemblages associated with both estuarine and marine ecosystems, with particular emphasis on ecological resilience of fish assemblages. The research team has long been dedicated to fish ecology in coastal and marine ecosystems, and is currently strongly contributing to progress in predictive capacities of models as well as in linking biodiversity to ecosystem function and services in order to contribute for conservation planning.

Author contributions: S.H., H.N.C. and R.P.V. conceived the ideas; S.H., I.C., M.L. and R.P.V. collected the data; P.C. developed the new method to assess the contribution of species to beta diversity; S.H. and R.P.V. conducted the data analyses; S.H. wrote the manuscript, which was carefully revised by P.C. and R.P.V.; R.P.V. supervised the work.

Editor: Michelle Gaither