

Supplementary Material for ‘Phylogenetic and functional evidence suggests that deep-ocean ecosystems are highly sensitive to environmental change and direct human disturbance’

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Detailed Methodology

1. Peracarid assemblage composition

312 box cores (USNEL type, area 0.25 m²) were collected from the Flemish Pass, the continental slopes of the Grand Banks and the slopes of the Flemish Cap (Northwest Atlantic Ocean) during spring and summer research cruises in 2009 and 2010 as part of the international NAFO (Northwest Atlantic Fisheries Organisation) potential vulnerable marine ecosystems – impacts of deep-sea fisheries (NEREIDA) programme. On-board the research vessel *Miguel Oliver*, box cores were drained of excess water and sediment subsamples were taken to a depth of 2 cm for particle size analysis and geochemical processing. The remaining sediment was split into 0-5 cm and deeper than 5 cm fractions and washed over a 0.5 mm mesh sieve. Material retained on this sieve was fixed in 4 % formaldehyde.

Initial processing of the box core samples (0-5 cm fraction) was undertaken at the Institute of Estuarine and Coastal Studies (IECS), University of Hull, UK; formaldehyde was drained over a 1 mm mesh sieve and retained specimens were

transferred to 70 % industrial methylated spirits (IMS). Specimens of peracarid crustacean were identified to genus by authors OSA and TH under Leica EZ5 and Motic SMZ-171T binocular microscopes, enumerated, labelled and stored in 80 % IMS.

2. Supertree construction

Phylogenetic relationships between all sampled genera were determined by the construction of a ‘supertree’ (Fig. 2a) (phylogenetic tree produced by the combination of a number of source trees with overlapping, but not necessarily identical taxon sets (1)). A supertree approach was necessary since this was the only available method capable of producing a single phylogeny describing the evolutionary relationships between all sampled peracarid genera.

2.1 Collation of published phylogenies

Google Scholar was used to search for all publications containing evolutionary trees (published up to 1st May 2016) that satisfied the following three criteria: 1) publications contain original trees, 2) trees contain two or more marine peracarid families, and 3) trees have been analysed using a recognised phylogenetic optimality criterion. This search yielded 59 studies. From these studies, a single tree for each unique combination of characters analysed (e.g. gene(s) used or morphological characters considered), taxa set analysed, weighting scheme employed and phylogenetic optimality criterion selected was retained for analysis. This step was taken in order to ensure that there was no duplication of source tree topology (which represents an emergent characteristic of source trees as phylogenetic hypotheses as well as the information directly used during supertree construction) in the analysed dataset that would otherwise unfairly weight the analysis as a result of pseudoreplication (1). Following application of these requirements, 127 evolutionary trees were retained for analysis (see Table S2).

Tree topologies were extracted from publications by taking screenshots. Images were cleaned to remove unnecessary background graphics using Microsoft Paint 6.1 and saved as JPEG files. TreeSnatcher Plus (2) was used to generate ‘Newick’ expressions of the tree topologies depicted in the images. These ‘Newick’ expressions were imported into Archaeopteryx 0.9901b (3, 4) where all monophyletic taxonomic groups were labelled (e.g. genera, families, orders) following World Register of Marine Species (WoRMS) systematic nomenclature (5). The labelled ‘Newick’ expressions were imported into Supertree Toolkit 2.14 (6) where details relating to the origin of each tree were added, and summary Nexus files were produced.

2.2 Construction of supertree topology

Supertrees were constructed using the program MultiLevelSupertree (MLS) 1.0 (7). MLS can accommodate source trees where terminal taxa are at varying taxonomic levels (i.e. nested source trees), and makes use of vertical overlap between source trees to inform tree construction.

MLS was run on the Oxford University Advanced Research Computing supercomputer ‘ARCUS (Phase B)’ (<http://www.arc.ox.ac.uk/content/home>). However, even using this high-performance computer, the run time for the complete dataset was prohibitively long, exceeding that permitted by the system. As such (and since the monophyletic status of amphipods, isopods, cumaceans and tanaidaceans is well supported by both morphological and molecular datasets (8-13)), supertree construction was split into five separate runs. Firstly, the program was run individually for Amphipoda, Isopoda, Tanaidacea and Cumacea using reduced datasets containing only those source trees that focus primarily on each of the taxa. For each of these runs a taxonomy tree was used to guide the program. For the case of Amphipoda, Isopoda and Tanaidacea, this taxonomy tree was down-weighted by ten times relative to the other input trees to ensure it did not have undue influence over the resulting supertree (which would negatively influence resolution). For the case of Cumacea, where fewer input trees were available, the taxonomy tree was down-weighted as far as possible (by four times relative to the other input trees). All other options were kept constant for each of these four runs, these being “treestransform = GeneralAB, ArcsWeight = 1, AllMC = true, Taxonomy = true, FromTaxonomyDeduction = true, PhyloTree = true, FreeAllVert = true, FullGraph = false” (see Berry et al. (7) and program documentation for a full explanation of these options).

Prior to undertaking the fifth run of supertree construction, a further two phylogenetic trees were constructed *de novo* using Maximum Likelihood (ML) and Bayesian methodology based on 18S SSU rDNA, 16S rDNA, cytochrome c oxidase 1 (COI) and Histone H3 gene sequences downloaded from GenBank (14) (see Supporting Appendix 1) in order to provide reference branch length data for the supertree. The topologies of these trees are available from the authors upon request. For peracarid families sampled by the NEREIDA box cores, all 18S, 16S, COI and H3 sequences available on GenBank were downloaded and collated using MEGA 7 (15). In cases where multiple copies of a gene were available per species, the longest available sequence was chosen. In addition, sequence data for a selection of malacostracan outgroup taxa were obtained from GenBank to root the resultant phylogenies in a broader taxonomic context.

Genes were aligned individually using MAFFT v7.273 (16) running on the MAFFT online server (<http://mafft.cbrc.jp/alignment/server/>). Sequences were first checked to confirm directionality using LAST plots produced by MAFFT. For protein-coding sequences (COI and H3), the G-INS-I alignment strategy was selected. This is an iterative method that is highly accurate for alignments of sequences with global homology. For 18S and 16S the E-INS-I alignment strategy was selected. This is also an iterative method, but is more appropriate for sequences with several conserved motifs that are embedded in long unalignable regions. In all cases default parameters were selected.

Alignments were scrutinised using trimAl v1.2 (17) in order to identify highly variable regions where alignments were unlikely to be reliable. This approach has been shown to improve signal to noise ratios, resulting in more reliable estimates of phylogeny (18). This resulted in the trimming of alignments from 7570 base pairs (bp) to 1271 bp for 18S, from 921 bp to 494 bp for 16S, from 1629 bp to 444 bp for COI, and from 422 bp to 327 bp for H3.

All missing data for taxa was coded as '?', and those taxa which lacked data for 50 % or more of the length of the alignment were removed. This final alignment (18S, 16S, COI and H3) was concatenated using SequenceMatrix 1.8 (19) and consisted of 285 taxa and 2586 bp.

PartitionFinder v1.1.1 (20) was used to select the most appropriate model of evolution and partitioning scheme for the concatenated alignment using the following settings: “branchlengths: unlinked, models of evolution: raxml/mrbayes (depending on phylogenetic program used subsequently), model selection: BIC (arguably the most appropriate metric of model performance (21, 22)), scheme: all”. For subsequent RAxML and MrBayes analyses, the most appropriate model of nucleotide substitution was found to be the General Time Reversible Model (23) + invariable site proportion + gamma-distributed rate variation among sites (GTR+I+G) applied across two partitions: 1) 16S, COI, and 3rd codon position of H3, and 2) 18S and 1st and 2nd codon positions of H3 (BIC score = 191772.795).

ML and Bayesian topologies were estimated using RAxML v8.2.8 (24) and MrBayes v3.2.6 (25), respectively, on the ‘CIPRES science gateway v3.3’ online server (26). RAxML-HPC v.8 on XSEDE was selected and all parameters were left as default other than the following: “Maximum hours to run: 12, Outgroup: *Nebalia_sp*, *Paranebalia_longipes* (the most basal members of the analysis), Mixed/partitioned model: specified as per PartitionFinder results, Estimate proportion of invariable sites: yes, Analysis type: Rapid bootstrap analysis/search for best-scoring ML tree, Model for bootstrapping

phase: GTRGAMMA, Bootstrapping type: rapid bootstrapping with RAxML halting bootstrapping automatically based on the autoMRE criterion”.

For the MrBayes run, MrBayes on XSEDE (3.2.6) was selected. Other than maximum hours to run (168 hours), all options were specified via a MrBayes data block. Partitions were set as per PartitionFinder results, with parameters unlinked and rates allowed to vary such that each of the two partitions could evolve under independent scenarios. Two runs of 100,000,000 generations were conducted, with trees being sampled every 2000 generations using eight Markov chains with a heating value of 0.12. Trace plots of log-likelihood scores were examined using Tracer v1.6 (27), and the two runs were considered to have converged after 95,720,000 generations when the standard deviation of split frequencies fell below a value of 0.01. A burn-in of 20 % was selected since this corresponded to the relative stabilisation of sample probabilities, and the remaining trees were used to construct a 50 % majority rule consensus tree.

The output trees of the four MLS runs and the ML and Bayesian analyses of the GenBank-derived sequence data were combined, using MLS, during the fifth round of supertree construction with all source trees focussing primarily on higher-level relationships within Peracarida and Malacostraca to produce the final supertree. Again, a taxonomy tree was employed to guide the program, and this was down-weighted by four times relative to the standard input trees, whilst the supertrees for Amphipoda, Isopoda, Tanaidacea and Cumacea, and the original ML and Bayesian trees derived from GenBank sequences were up-weighted by 4.5 times (maximally) relative to the standard input trees. All other options were left as above. The resulting supertree has 1487 terminal taxa.

2.3 Mapping of branch lengths onto supertree topology

Supertree topology was trimmed to include only those taxa present in the GenBank alignment. Maximum likelihood branch lengths were estimated for this topology using RAxML v8.2.8 (24) running on the ‘CIPRES science gateway v3.3’ online server (26). RAxML-HPC v.8 on XSEDE was selected and all parameters were left as default other than the following: “Maximum hours to run: 12, Outgroup: *Nebalia_sp*, *Paranebalia_longipes*, Constraint tree: Yes (supertree topology trimmed to only taxa present in the GenBank alignment), Mixed/partitioned model: specified as per PartitionFinder results, Estimate proportion of invariable sites: Yes, Analysis type: Rapid hill-climbing algorithm, Model for bootstrapping phase: GTRGAMMA”.

Next, following Strauss et al. (28), common nodes between the supertree and ML branch length tree were labelled using the ‘COMNODE’ function of PhyloCom 4.2 (29). This labelled ML branch length tree was used as an input for the program R8s 1.8 (30) in order to obtain a chronogram of taxa relationships. 22 nodes were constrained with age estimates based on fossil data (see Table S1) to calibrate the chronogram. The root node of the phylogeny (representing Malacostraca) required a maximum age constraint for divergence times to be estimated, and this was set to 550 Ma (million years before present) based on evidence from fossil data and previous molecular sequence-based estimates (31-34). Non-Parametric Rate Smoothing (NPRS) with Powell optimisation was selected as the analysis method. NPRS is a non-parametric method that relaxes the stringency of molecular clock assumptions, being particularly appropriate in cases of relatively high rate variation among lineages (35). 10 runs of the program were conducted, with 20 perturbed restarts per run and a maximum of 100,000 iterations permitted in order to provide an effective search strategy that is most likely to terminate at the global optimum (30). The CheckGradient option was specified as an additional check that the global optimum had been found.

Following this, the BLADJ function of PhyloCom 4.2 (29) was used to set the common labelled internal nodes between the supertree and dated R8s chronogram to the same ages, and then interpolate these ages across the remainder of the supertree to obtain a fully-dated phylogeny. This method is influenced by taxon sampling, and the GenBank sequences targeted do show gaps in coverage across the supertree. However, the gaps occurred, in general, in regions of the supertree not relevant to this study – e.g. amongst shallow-water, freshwater and terrestrial taxa which do not occur in the box core samples, including Talitrida, Crangonyctidira, Oniscidea and Sphaeromatidea.

3. Functional dendrogram construction

Functional similarities between sampled taxa were investigated by the production of a functional dendrogram (Fig. 2b) based on the relative scoring of sampled families for a selection of traits. Functional traits were characterised at family-level (except for Tanaidacea ‘*incertae sedis*’, which had to be scored at genus level) because of a paucity of data at higher taxonomic resolutions. Trait groupings and traits (see Table S3) were chosen based on ecological relevance and data availability. A fuzzy coding (36) method was utilised for trait scoring to enable the coding of variability in trait scores within a family/individual, whereby a score of 0 reflects no affinity of the taxon with the trait in question, and a score of 5 represents complete affinity. To ensure equal weighting of trait groupings, the total score for a taxon for each trait group was set to 5.

Where continuous trait data were available for families (e.g. the trait groupings ‘maximum body length’ and ‘maximum fecundity’), natural breaks suitable for splitting the data into distinct categories were identified via k-means clustering (37) (Sturges’ formula used to determine an appropriate number of categories (38)) in R 3.0.2 (39) using the package ‘classInt’ 0.1-22 (40), based on the maximal values for each family.

Trait scorings (77 taxa, 38 traits in 10 trait groupings) were based on available literature where possible (93 sources, 58.0 % of trait groupings). In cases where literature was insufficient, taxa were scored based on the expert opinion of the authors (TH, AB, GJB, SG and OSA) (42.0 % of scores).

The trait database was converted into a dendrogram describing functional similarity between taxa via hierarchical clustering methodology. Following Petchey and Gaston (41) and Mouchet et al. (42), the most appropriate distance metric and clustering method was selected by comparison of cophenetic correlation coefficient values (43). Using the methodology of Mouchet et al. (42), Gower and Euclidean distance metrics, and the following clustering methods, were analysed: single linkage, complete linkage, unweighted pair group method using arithmetic averages (UPGMA), weighted pair group method using arithmetic averages (WPGMA), unweighted pair group centroid method (UPGMC), weighted pair group centroid method (WPGMC), and Ward’s. Further, all potential consensus tree combinations were analysed, giving a total of 254 combinations of distance and clustering methods. Analyses were conducted in R (39) using a script provided by Dr Maud Mouchet. The distance and clustering method that was found to perform best was Euclidean distance and UPGMA clustering, and these methods were utilised to produce the functional dendrogram using the R package ‘stats’ (39).

4. Testing for phylogenetic and functional assemblage structure

4.1 Phylogenetic assemblage structure

Phylogenetic assemblage structure was investigated across all box core samples and across seven data subsets, each consisting of 30 box cores chosen at random from within a set radius (50, 100, 200, 300, 400, 500 and 600 km) of the central-most sampling point, using the constructed supertree (Fig.2a) and genus-level peracarid assemblage matrix. The central-most box core was identified using the ‘Central Feature’ tool (Euclidean distance), and buffers were created using the ‘Buffer’ tool, in ESRI ArcGIS 10.1. Assemblage matrices were reduced to contain only those box cores which had two or more peracarid taxa present. Box core spatial subsets were produced because the dominant processes influencing assemblage phylogenetic/functional structure can appear to change depending on the spatial scale investigated (44). The

phylostruct function of the R package ‘picante 1.6-2’ (45) was used to test for phylogenetic assemblage structure. ‘Phylogenetic Species Variability’ (PSV) (46) was calculated for each box core retained in the dataset based on a phylogenetic covariance matrix describing anticipated covariance between the sampled genera in the values of a hypothetical trait under ‘Brownian motion’ evolution. This provides a means to translate a phylogeny into a statistical measure of phylogenetic relatedness (46-48). PSV was selected as the metric of phylogenetic dispersion since it represents a pure measure of phylogenetic signal not confounded by species richness or abundance. It also has advantageous statistical properties when used in permutation tests for phylogenetic structure (46, 48). Permutation tests were used to determine whether the average PSV value of all box cores, and box core subsets, was significantly different from that expected given two null hypotheses – ‘Null 1’ and ‘Null 2’ – (see Helmus et al. (46)). ‘Null 1’ assumes that each box core sample consists of a random draw of taxa from the available taxa pool – i.e. no phylogenetic structure is present in either taxa prevalence (the number of box cores each taxon is present in), or assemblage composition (the co-occurrence of taxa in a box core after taking into account their prevalence) (48). ‘Null 1’ achieves this by randomising taxon presence within box core samples (45). ‘Null 2’ assumes that each box core sample consists of a random draw of taxa from the available taxa pool given their prevalence among the box cores – i.e. there is no phylogenetic structure present in sample composition (48). It achieves this by randomising taxon occurrences between box cores (45). By testing both hypotheses, one can determine whether any phylogenetic assemblage structure is the result of differences in the prevalence of taxa, or differences in the probability of co-occurrence of related taxa. The box core assemblage matrix was permuted under each of these null hypotheses to give 100000 permutation datasets. For each permutation dataset, average PSV was calculated, generating a null distribution against which the average box core PSV value could be compared.

4.2 Functional assemblage structure

Functional assemblage structure was investigated both across all box core samples, and within the subsets of samples, using the constructed functional dendrogram (Fig. 2b) and family-level peracarid assemblage matrix. Since functional dendrograms and phylogenies are analogous in form (41) they can be analysed using identical mathematical methodology. Thus, identical methodology was employed as outlined above for the testing of phylogenetic assemblage structure.

5. Testing for phylogenetic signal

In order to explicitly test for significant phylogenetic signal, defined as the tendency for related taxa to phenotypically resemble each other more than they resemble taxa drawn at random from the taxa pool (49, 50), we applied a Mantel test. The Mantel test (51) is a statistical method to evaluate the similarity between two distance matrices (52). In this case these

distance matrices represent the phylogenetic and functional distances between taxa. Unlike other tests for phylogenetic signal (53, 54), Mantel tests are able to estimate phylogenetic signal across multiple traits simultaneously, and when used appropriately, represent a powerful method to detect phylogenetic signal (50). Following Hardy and Pavoine (55) and based on the supertree of Peracarida (Fig. 2a), we calculated the square root of patristic distance as a measure of phylogenetic distance between taxa using the R package 'ape 4.1', whilst Euclidean distance was calculated as a measure of trait similarity between taxa based on the peracarid functional trait matrix (see Supplementary Material Dataset 3). The Mantel test was performed using the R package 'vegan 2.0-9' (100000 permutations).

In order to assess the strength of phylogenetic signal in individual traits, following Munkemuller et al. (49) and based on the supertree of Peracarida (Fig. 2a), we calculated Pagel's λ (53) for each trait in the peracarid functional trait table (Supplementary Material Dataset 3). Pagel's λ was calculated in R 3.0.2 (39) using the package 'phylosignal 1.2' (56) (100000 permutations).

6. Characterisation of the physical environment

6.1 Bathymetry

The location (latitude and longitude) of each box core sample was recorded as collected using the Global Positioning System of the research vessel *Miguel Oliver*. Depth (to the nearest metre) was extracted for each box core location following multibeam bathymetric surveys using a Kongsberg EM 302 30 kHz system. Multibeam bathymetry was gridded to a 75x75 m (5625 m²) cell size using ESRI ArcGIS 10.1, and a five-cell neighbourhood mean filter was applied to remove artefacts introduced by the gridding process.

Further bathymetric variables were derived from this bathymetric grid by Anna Downie (Cefas, Lowestoft, UK) using the Spatial Analyst extension of ESRI ArcGIS 10.1. These included slope, eastness and northness, roughness (3x3 cell window) and standard deviation of bathymetry values (3x3 cell window). Benthic Terrain Modeller (57) was used to calculate Bathymetric Position Index (BPI) over a range of radii (outer radii of 25, 50, 75, 100, 125 and 150 cells, inner radius of 1 cell in all cases) as well as seafloor rugosity (5x5 and 25x25 cell windows), these data also being provided by Dr Anna Downie.

6.2 Fishing intensity

Bottom trawling effort was quantified based on the conversion of time-referenced VMS signal locations (collected from 2008 to 2012 and provided by NAFO) into trawl paths. Only VMS records indicating boat speeds of between 0.5 and 5 knots (inclusive) were utilised as these are typical of active deep-sea bottom trawling operations (58-60). Individual trawl paths were identified based on boat identity, speed, location, date and time. The Points to Line Tool in ArcGIS 10.1 (Data Management) was used to convert the point dataset into a series of trawl paths, and the Line Density Tool (Spatial Analyst extension) was used to measure the total length of trawls per km² within a set radius (1, 3, or 5 km (60)) from each box core. These values were extracted using the Extract Values to Points tool in the Spatial Analyst extension, with local interpolation option selected.

6.3 Geological context

The sediments of the slopes of the Flemish Cap, Flemish Pass and Grand Banks were classified into the following geological categories based on their acoustic characteristics, depth and slope (determined using Kongsberg EM 302 30 kHz multibeam bathymetric survey and TOPAS PS018 parametric sub-bottom profiler data): shelf-indenting canyon, deep-water canyon (canyon head >400 m water depth), inter-canyon or inter-channel ridge, flat area with abundant iceberg scours and pits, gully, steep flank (>6.4 ° slope), thick sediment draped over irregular seafloor, sediment wave, sediment drift, smooth area without evidence of erosion, failed seabed, and rough topography of mass transport debris (MTD) (61). The category relating to the location of each box core was recorded, using ESRI ArcGIS 10.1 for data visualisation.

6.4 Sediment Particle Size Analysis

Sediment particle size analysis was undertaken on box core subsamples (2 cm depth) at the Bedford Institute of Oceanography, Halifax, Canada, in the Geological Survey of Canada (Atlantic) Core Processing Laboratory by Owen Brown and colleagues using a Beckman Coulter LS 230 Laser Diffraction Particle Size Analyser (capable of measuring particles of size 0.04-2000 µm) (62). Percent clay/silt/sand was calculated for each core subsample based the following particle size categories consistent with the 'Phi' (Φ) scale of Krumbein (63) – clay: 0.375 µm to <4.241 µm, silt: 4.241 µm to <63.41 µm, sand: 63.41 µm to <2000 µm. Particle size diversity was calculated from the percent dry weight of each Φ size class (12 size classes between -1 and >10 Φ) using the Shannon-Wiener diversity index (calculated in R 3.0.2 (39) using the package 'vegan 2.0-9' (64)) and following Etter and Grassle (65) and Leduc et al. (66).

6.5 Sediment carbon content

Analysis of the carbon content of box core sub-samples was undertaken at the Bedford Institute of Oceanography, Halifax, Canada, in the Geological Survey of Canada (Atlantic) Core Processing Laboratory by William Leblanc and colleagues (62). Total carbon and organic carbon content were determined using a Leco TruSpec CHN analyser, model number 630-100-400. Samples were dried at 60 °C and ground to a fine homogenous powder prior to analysis. To measure organic carbon, carbonate carbon was first removed from the dried ground samples with dilute acid: approximately 2-3 g of sediment was placed in a 50 mL Falcon tube with 15 mL of 10 % HCl. This was shaken vigorously using a Vortex-Genie mixer and the sample was left overnight to ensure completion of reaction. The solution was decanted and replaced with 15 mL of distilled water before being shaken again in the vortex mixer. More distilled water was added to bring the total volume in the tube to 50 mL. After settling, the distilled water was decanted and the previous step repeated twice more. Following this, samples were dried completely at 60 °C before being ground once more. Once the carbonate carbon had been removed, 100 mg of each sample was weighed into a tin foil boat for analysis in the CHN analyser. Inorganic carbon was determined by the difference between the total carbon and organic carbon measurements for each sample (62).

6.6 Chlorophyll *a* and Particulate Organic Carbon concentrations

Observed surface chlorophyll *a* and particulate organic carbon (POC) concentrations (mg/m^3) for the study area were obtained from the Giovanni ocean colour radiometry online data system (accessible at <https://giovanni.gsfc.nasa.gov/giovanni/>). Latitude - longitude time averaged MODIS AQUA 4 km resolution data was downloaded for the years 2008, 2009 and 2010, and this was converted into a raster file in QGIS 2.2. All values smaller than zero were masked and the data were interpolated to 2500x2500 pixels (525 m resolution) using the Inverse Distance Weighting method. Values at box core locations for the year prior to sample collection (to account for time lag between production and delivery to the seafloor) and the sample collection year were extracted by Dr Jesse van der Grient using the Point Sampling Tool in QGIS 2.2 and averaged. POC delivery to the seafloor was calculated from surface POC concentrations following region-specific equations developed by Lutz et al. (67). Equations calibrated using observed POC and radiochemically corrected POC values (which aim to correct for under-sampling of POC flux within the mesopelagic zone) were utilised to calculate POC transport to the depth of each box core. Since no equation specific to the study region was available, equations calibrated to the Northeast Atlantic and Sargasso Sea were used.

6.7 Oceanographic variables

Seafloor temperature and salinity values were measured at each box core location using a Sea-Bird Electronics 25 Sealogger CTD. This information was incorporated into a modelled monthly average seafloor temperature and salinity data layer (1/12 degree resolution, data between 1990 and 2012) for the whole Flemish Cap, Flemish Pass and Grand Banks area (produced by Wang and Greenan (68), Bedford Institute of Oceanography, Halifax, Canada). Such a layer was also available for seafloor meridional and zonal current speeds (68).

For temperature, salinity and meridional/zonal current speeds, seafloor monthly values were extracted for the study area and averaged across the year prior to box core collection, and across the year of box core collection. These layers were converted to raster files in QGIS 2.2 by Dr Jesse van der Grient before interpolation to 3000x3000 pixels (578 m resolution) using the Inverse Distance Weighting method. Values were extracted at box core locations using the Point Sampling Tool of QGIS 2.2. Absolute current speed was calculated from the extracted meridional and zonal current velocity values using Pythagoras' theorem. These data were then summarised as values for the box core collection year, and an average value for the box core collection year and the previous year (since the typical lifespan of a peracarid crustacean is one to two years (69)). Further, 10 year minimum/maximum values for temperature and current speed, respectively, and 10 year average values for both variables were calculated across the study area by Dr Anna Downie (Cefas, Lowestoft, UK) based on the oceanographic model of Wang and Greenan (68) to capture longer-term variability. These layers were interpolated to a 75x75 m grid using the Empirical Bayesian Kriging tool in the Geostatistical Analyst extension of ESRI ArcGIS 10.1 and extracted at box core locations using the Extract Values to Points tool in the Spatial Analyst extension of ArcGIS 10.1.

7. Statistical analyses

7.1 Initial variable selection

The environmental dataset was first refined to remove all highly correlated variables, since high multicollinearity can have severe effects on the estimation of model parameters (70). This was achieved by a two-step process. First, where several variables were available for a single facet of environmental variation (e.g. the case of fishing intensity), the variable in that group with the highest Variance Inflation Factor (VIF) was selected to represent that environmental parameter (since the variable with the highest VIF value will best capture the information present in the alternative variables). Secondly, a round of VIF calculations was undertaken amongst all selected variables from the first step. Variables were removed from the analysis in a stepwise manner (those with highest VIF first) until all variables had a VIF value less than 5 - an accepted cut-

off (71). The resulting dataset contained 19 variables describing the physical environment (see Table S4). All VIF calculations were undertaken in R 3.0.2 (39) using the package ‘HH 3.1-32’ (72).

7.2 Construction of Generalised Additive Models (GAMs)

Because initial data exploration revealed a high number of relationships between dependent and independent variables that appeared to be non-linear, individual GAMs were constructed in R 3.0.2 (39) using the package ‘mgcv 1.7-26’ (73) to determine which combination of the 19 physical environmental variables most effectively explained variance in PSV and FSV between box cores. Prior to analysis, trawling intensity (3 km radius) was transformed ($\text{Log}_{10}(x + 1)$) to reduce skew in the data. Original measurement scales were retained for all other variables because data distributions were deemed acceptable and to simplify the interpretation of model outputs. Further, all box cores with zero abundance of peracarids, and box core 341 (with only one peracarid genus present), were removed.

Full GAMs consisted of all variables contained within Table S4, with smoothers added to all continuous variables. The most appropriate error distributions and link functions were selected by the consideration of model diagnostics and the Akaike Information Criterion (AIC) (74) (PSV = Gaussian (identity); FSV = Gamma (identity)), and acceptable satisfaction of model assumptions (e.g. normal distribution of residuals and homogeneity of variance of residuals) was investigated using the *gam.check* function of the package ‘mgcv 1.7-26’ (Wood, 2013a). A penalised thin-plate regression spline was used as the smoothing function, and smoothing parameters were optimised automatically on the basis of the Generalised Cross Validation criterion (73).

The explanatory terms included in each GAM were refined from the full model via backwards stepwise selection by consideration of variable *P*-values and model AIC until a minimum AIC value was reached. At this point, the model was considered optimal.

Table S1 | Details of nodes constrained with age estimates based on fossil data in order to calibrate the supertree chronogram

Node	Minimum age of divergence (Ma)	Strata	Reference
Phyllocarida - other Malacostraca	489.5	Jiangshanian	(32)
Hoplocarida - Euphausicea	313.0	Moscovian	(75)
Peracarida - other Malacostraca	358.9	Famennian	(76)
Lophogastrida - other Peracarida	307.0	Moscovian	(77)
<i>Eucopia</i> - <i>Gnathophausia</i>	163.5	Callovian	(78)
Mysida - other Peracarida	163.5	Callovian	(78)
'Gammaridae - type' - other Gammaroidea	45.0	Lutetian	(79)
Phreatoicoidea - Asellota	307.0	Moscovian	(80)
Cymothoidea - Valvifera	166.1	Bathonian	(81)
Cirolanidae - Anthuroidea	170.3	Aalenian	(82)
<i>Cirolana</i> - other Cirolanidae	125.0	Barremian	(83)
Tanaidacea - other Peracarida	326.4	Serpukhovian	(84)
Apseudomorpha - Tanaidomorpha	170.3	Aalenian	(85)
Cumacea - other Peracarida	323.2	Serpukhovian	(86)
Diastylidae - Bodotriidae	252.2	Changhsingian	(87)
Pleocyemata - other Decapoda	358.9	Famennian	(88)
Brachyura - other Pleocyemata	185.0	Pliensbachian	(89)
Astacidae - other Astacoidea	145.0	Tithonian	(90)
Penaeidae - other Dendrobranchiata	145.0	Tithonian	(91)
Atyoidea - Bresilioidea	125.0	Barremian	(92)
Stenopodidea - Axiidea	93.9	Cenomanian	(93)
Chirostyloidea - Hippoidea	66.0	Maastrichtian	(94)

Table S2 | Source literature for supertree construction

Source	Figure number(s)
Araujo-Silva and Larsen, (95)	1
Best and Stachowicz, (96)	1, 2a, 2b
Bird and Larsen, (97)	1
Błażewicz-Paszkowycz et al. (98)	9
Boyko et al. (99)	1, 2
Brandt and Poore, (100)	6b, 6d
Browne et al. (101)	S1, S2, S3, S4, S5
Brusca and Wilson, (102)	14
Bybee et al. (103)	2
Corrigan et al. (104)	1, 2, 4
Dreyer and Wagele, (105)	5, 8, 9
Dreyer and Wagele, (106)	6, 8
Drumm, (13)	1a, 1b, 2a, 2b, 3
Englisch et al. (107)	1, 3, 4, 5, 6
Guerrero Kommritz and Brandt, (108)	2
Havermans et al. (109)	1a, 1b
Haye et al. (110)	1, 3b
Held, (111)	6
Hou and Sket, (112)	1, 2
Hurt et al. (113)	2a, 2b
Ito et al. (114)	2a, 2b, 2c
Jenner et al. (115)	1, 2a, 2b, 2c, 2d, 5, 6a, 6b, 7a, 7b
Kakui et al. (116)	2, 3, 4, 5
Kakui et al. (117)	7a, 7b
Kavanagh and Wilson, (118)	3b
Ki et al. (119)	1b
Kilpert et al. (120)	2
Kim and Kim, (121)	5
Koenemann et al. (122)	1, A4, A7
Krapp-Schickel and Koenemann, (123)	4, 5, 7, 9
Larsen and Wilson, (124)	1
Larsen and Shimomura, (125)	12
Larsen et al. (126)	1a, 1b, S1, S2
Larsen and Araujo-Silva, (127)	1
Lins et al. (128)	S1, S2, S3
Lorz and Held, (129)	1, 2, 3, 4, 5
Lowry and Myers, (130)	10
Meland and Willassen, (11)	2
Myers and Lowry, (131)	12
Poore, (132)	4a
Raupach et al. (133)	1
Raupach et al. (134)	1, 2, S1
Raupach et al. (135)	2, 4b
Rehm, (136)	1
Riehl et al. (137)	26
Ritchie et al. (138)	1
Serejo, (139)	1
Shen et al. (140)	3
Souza-Filho and Serejo, (141)	7
Spears et al. (10)	2b, 3b, 4b
Takeuchi, (142)	1
Verheye et al. (143)	2, 3, 3b
Wagele et al. (144)	2, 3
Wetzer, (145)	1, 2, 5, 6, 7
Wetzer et al. (146)	3
White and Reimer, (147)	1
Wilson, (148)	3a, 3b
Wilson, (12)	2, 3, 5
Wirkner and Richter, (149)	2

Table S3 | Trait groupings and trait modalities used to quantify functional attributes of sampled peracarid families

Trait grouping	Trait modalities	Comments
Maximum adult length	Very small (0 to <4.7 mm)	Maximum adult length attained. Categories based on K-means clustering of continuous data
	Small (4.7 to <9.5 mm)	
	Medium (9.5 to <22.2 mm)	
	Large (22.2 to <45 mm)	
	Very large (>45 mm)	
Fragility	Fragile	Typically missing antennae, some pereopods and uropods upon collection - may be dis-articulated
	Medium	Typically with damaged antennae, some incomplete pereopods, and may have lost uropods upon collection
	Robust	Essentially complete specimens upon collection
Maximum fecundity	Very low (0 to <18)	Maximum number of eggs produced in a single brood. Categories based on K-means clustering of continuous data
	Low (18 to <31)	
	Medium (31 to <65)	
	High (65 to <135)	
	Very high (>135)	
Feeding group	Deposit feeder	Semi-selective benthic particle feeder
	Suspension feeder	Semi-selective suspended particle feeder
	Active predator (including foraminifera)	Active selection of live prey
	Scavenger	Opportunistic feeder on recently dead material
	Parasitic	Prolonged feeding from another live organism in a non-mutually beneficial manner
Habitat space	Infaunal	Below substratum/water interface
	Epibenthic	On surface of substratum
	Benthopelagic	Near bottom/pelagic habitat
	Epibiotic (benthic)	Living on another benthic organism
Living habit	Free	Free moving
	Tube/burrow dweller	Use of constructed tube/burrow
	Parasite/direct commensal	Close relationship with another organism (obligate or facultative)
Motility	Fast swimmer	Able to make rapid, significant progress through water e.g. as an escape mechanism
	Slow swimmer/crawler	Able to swim, but not at a significant speed. Able to move by crawling across substratum
	Crawler/limited swimmer	Movement predominantly by crawling across substratum. Are able to move in the water column if necessary

Trait grouping	Trait modalities	Comments
	Crawler (no swim)	Constrained to crawling across substratum
	Burrower	Able to burrow into and make way through substratum
Bioturbation/sediment transport potential	Limited	Limited sediment movement capacity
	Low-level incidental diffusive mixing	Incidental sediment transport/mixing e.g. by movement of limbs across substratum
	Active significant sediment transport	Significant sediment transport/mixing due to active working of sediment e.g. burrowing
Sociability	Solitary	Lives individually
	Gregarious	Lives in groups
	Swarming	Can congregate in large abundances
Parental care	Limited	No interaction with young following release from brood pouch
	Extended	Active feeding and/or defence of young following release from brood pouch

Table S4 | Details of the environmental variables retained for analysis following Variance Inflation Factor variable selection

Environmental facet	Representing variable
Short-term temperature	Modelled seafloor temperature – average for year box core collected (°C)
Long-term temperature	Modelled seafloor temperature – average for 10 years prior to box core collection (°C)
Short-term current speed	Modelled seafloor absolute current speed – average for year box core collected (m/s)
Long-term current speed	Modelled seafloor maximum current speed – maximum value for 10 years prior to box core collection (m/s)
Surface productivity	Surface chlorophyll <i>a</i> - MODIS average for year box core collected plus previous year (mg/m ³)
Energy availability at seafloor	Surface particulate organic carbon – MODIS average for year box core collected plus previous year (mg/m ³)
	Particulate organic carbon at seafloor - Sargasso sea equation (not radionuclide corrected) of Lutz et al. (67) applied to MODIS surface particulate organic carbon average for year box core collected plus previous year (mg/m ³)
	Percent total carbon content in top 2 cm of box core
Sediment grain size	Percent organic carbon content in top 2 cm of box core
	Percent sand content in top 2 cm of box core
Geological environment	Shannon sediment particle size diversity in top 2 cm of box core
	Geological environment category
Fishing intensity	Trawl density – Total trawl length per km ² of seafloor between 2008 and 2012 within a 3 km radius of the box core location (Log ₁₀)
Seafloor topography	Bathymetric position index – value for 125 cell (9375 m) radius window around box core location
	Seafloor rugosity – value for a 25x25 cell (1875x1875 m) window around box core location
	Seafloor roughness – value for a 3x3 cell (225x225 m) window around box core location
Annual and seasonal variation	Collection year – year box core collected (categorical)
	Collection month – month box core collected (categorical)
Variation in collection procedure	Crew identity at time of box core collection (categorical)

Table S5 | Pagel's λ and associated P -value (based on supertree of Peracarida) for trait modalities used to quantify functional attributes of sampled peracarid families. $P < 0.05$ suggests that trait evolution is indistinguishable from that expected under a pure Brownian model of evolution (i.e. significant phylogenetic signal).

Trait grouping	Trait modalities	Pagel's λ	P -value
Maximum adult length	Very small (0 to <4.7 mm)	0.910	<0.0001
	Small (4.7 to <9.5 mm)	0.673	0.0005
	Medium (9.5 to <22.2 mm)	1.163	<0.0001
	Large (22.2 to <45 mm)	1.163	<0.0001
	Very large (>45 mm)	1.163	<0.0001
Fragility	Fragile	0.749	0.0030
	Medium	0.868	0.0205
	Robust	0.519	0.0561
Maximum fecundity	Very low (0 to <18)	0.302	0.0262
	Low (18 to <31)	<0.001	1.0000
	Medium (31 to <65)	1.163	<0.0001
	High (65 to <135)	<0.001	1.0000
	Very high (>135)	0.098	0.5987
Feeding group	Deposit feeder	1.143	<0.0001
	Suspension feeder	1.163	<0.0001
	Active predator (including foraminifera)	1.113	0.0014
	Scavenger	1.163	<0.0001
	Parasitic	1.163	<0.0001
Habitat space	Infaunal	1.063	<0.0001
	Epibenthic	1.046	<0.0001
	Benthopelagic	1.163	<0.0001
	Epibiotic (benthic)	1.163	<0.0001
Living habit	Free	1.085	<0.0001
	Tube/burrow dweller	1.089	<0.0001
	Parasite/direct commensal	1.163	<0.0001
Motility	Fast swimmer	1.163	<0.0001
	Slow swimmer/crawler	1.163	<0.0001
	Crawler/limited swimmer	1.163	<0.0001
	Crawler (no swim)	1.163	<0.0001
	Burrower	1.163	<0.0001
Bioturbation/sediment transport potential	Limited	1.163	<0.0001
	Low-level incidental diffusive mixing	0.939	<0.0001

Trait grouping	Trait modalities	Page's λ	<i>P</i>-value
	Active significant sediment transport	0.963	<0.0001
Sociability	Solitary	1.133	<0.0001
	Gregarious	1.121	<0.0001
	Swarming	1.163	<0.0001
Parental care	Limited	0.870	0.0003
	Extended	0.870	0.0003

Additional files

Supporting Appendix 1:

Details of taxonomic identity and accession number for all GenBank sequences analysed.

Dataset 1: Peracarid assemblage abundance matrices (genus abundance by box core and family abundance by box core).

Dataset 2: Biological/ environmental variables matrix (Matrix of all biological ['PSV' and 'FSV'] and environmental variables analysed by box core).

Dataset 3: Functional trait matrix (Matrix of functional traits by peracarid family).

Dataset 3 key: Key to references cited in Dataset 3.

Dataset 4A: 'Newick' format file describing the presented supertree of Peracarida.

Dataset 4B: 'Newick' format file describing the presented functional dendrogram of Peracarida.

Above datasets and supporting appendix are also available at Figshare (DOI: 10.6084/m9.figshare.5858592; <https://figshare.com/s/afb47eafaab24bf18863>).

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