Department of
Biodiversity, Conservation
and Attractions

Development of a Monitoring Program for Benthic Infauna at Roebuck Bay and Eighty Mile Beach

WRM
Wetland Research & Management

Final Report

28 April 2021


Development of a Monitoring Program for Benthic Infauna at Roebuck Bay and Eighty-Mile Beach

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Frontispiece: Lumbrineridae polychaete (left), sampling benthos by hovercraft (centre), and a *Diopatra* polychaete (right) (photos Angela Rossen/Chris Glasby).

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1.0 INTRODUCTION

Eighty Mile Beach (EMB) and Roebuck Bay (RB), in the Kimberley Region in northwest Western Australia, are Ramsar-listed wetlands (Figures 1 & 2). They are recognized for supporting a high abundance of migratory shorebirds, and are two of the most important non-breeding areas for migratory shorebirds in the entire East Asian-Australasian flyway. There is increasing evidence of declines in most species of shorebird in the East Asian – Australasian flyway (*e.g.* Amano 2010, Wilson *et al.* 2011, Clemens *et al.* 2016, Hansen *et al*. 2016, Studds *et al.* 2017). These declines are likely to be driven largely by habitat loss at migratory staging areas on the east coast of Asia, but other factors, including habitat quality in Australian non-breeding grounds may also play a role (Clemens *et al.* 2016, Studds *et al.* 2017).

The large shorebird populations of EMB and RB are supported by a diverse and abundant benthic infauna residing in the inter-tidal mudflats (Piersma *et al.* 2016, Compton 2017). The diversity and biomass of this benthic infauna is considered critical to supporting the abundance of migratory shorebirds. The importance of this benthic infauna was first recognised in 1996, and resulted in a series of large-scale benthic infauna surveys, conducted at RB in 1997, 2000, 2002, 2006 and 2016 (Pepping *et al*. 1999, Piersma *et al.* 2016) and at EMB in 1999 and 2016 (Honkoop *et al.* 2008, Piersma *et al.* 2016). In addition, a long-term, initially monthly sampling program was initiated in 1997 at two locations in RB, Fall Point and One Tree (MonRoeb), with data from 1997 to 2005 analysed and reported (de Goeij *et al.* 2008).

These two sampling programs have provided a substantial volume of data which may be used to further our understanding of the ecology of these two wetlands. The large-scale benthic infauna surveys are based on a systematic random grid-based sampling design, providing comprehensive coverage of the two wetlands, and potentially providing high statistical power for determining species abundances. In the case of Roebuck Bay, an additional advantage is that multiple expeditions have been conducted over time. This provides a means of assessing the variability in species abundances and community composition across the bay and over time (spatial and temporal coverage). Furthermore, in the majority of these surveys, sampling was conducted to the highest taxonomic resolution possible, often with leading taxonomists joining the expeditions (Marc Lavaleye, NIOZ, pers comm.), making it possible to also assess effects of taxonomic resolution on the data outputs.

The monthly monitoring program (MonRoeb) provides data that may be used to assess seasonal and yearly changes in the benthos of RB at two locations, using replicated sampling. The design also allows an assessment of effects of varying levels of replication on statistical power (*i.e.* ability to detect change should it occur), and provides some, although limited, overlap with the large-scale surveys, with this overlap being invaluable for assessing constancy in patterns described by MonRoeb compared with the larger spatial scale.

Monitoring the ecological condition of Ramsar-listed wetlands is an obligation under the Ramsar Convention, and is necessary to ensure the ecological character of each site is maintained, and thereby the site can continue to support abundant shorebirds. It is also important to monitor sites in Australia regularly to pinpoint the causes of shorebird declines, and to assess whether population changes in shorebirds at a site level are consistent with national trends. If they are not, then it may be possible to identify local driving factors and assess if they can be corrected by local management.

The Department of Biodiversity, Conservation and Attractions (DBCA) is the Western Australian State department responsible for management of the State's Ramsar wetlands on behalf of the Commonwealth Government. To assist management of these two Ramsar sites, DBCA, with funding from BHP, contracted Wetland Research and Management (WRM) to design a robust program to monitor the ecological health of the two sites using the benthic infauna. Both the large-scale grid sampling and the MonRoeb data were to be used as a basis for designing a new monitoring program for RB and EMB. The MonRoeb data could be used to assess the seasonal and yearly variability of benthos in the bay and how representative the

MonRoeb sampling locations are of the wider RB surveys. Whereas the large-scale sampling could be used to examine the spatial structure of invertebrate assemblages across the sites, how assemblages vary temporally, what factors may be structuring these assemblages, and determine the frequency at which sampling needs to be conducted, the number of stations to be sampled, the number of samples to be taken and the level of taxonomic resolution needed to examine temporal trends.

In the first instance, the Royal Netherlands Institute for Sea Research (NIOZ), who have been pivotal in past work on these two sites, was subcontracted by WRM to analyse all the historical data collected for RB and EMB to design a monitoring program. Their specific tasks were to:

1. Conduct a detailed analysis of all existing data to gain a better understanding of spatial and temporal patterns in benthic infauna of Roebuck Bay and Eighty Mile Beach;
2. Use these analyses to design an ongoing monitoring program of benthic fauna of Roebuck Bay, optimising number of sites, locations for sites, levels of replication and timing of sampling.

The NIOZ report (Compton 2017) analysed aspects of the data, describing i) seasonality in the MonRoeb data, ii) between-survey differences in overall species lists, iii) effects of progressively increasing sampling grid size on data collected, and iv) a higher-level modelling of the spatial structure in the invertebrate fauna across RB in relation to available physico-chemical data, but with a low predictive capability. The report did not include detailed multivariate analyses to describe spatial and temporal changes in assemblages within and between the two sites, or test empirical relationships with physico-chemical parameters. Neither did it address the requirement for an objective monitoring design that was empirically based, nor include basic aspects of a robust monitoring program, such as the number of sites to sample, when to sample, where in each system to sample, the level of replication required for given effects sizes, and influence of level of taxonomic resolution, with a range of options to fit different budgets.

To address aspects not detailed by Compton (2017), the current report builds on the outputs of Compton (2017), and specifically addresses the requirements for a monitoring program that is empirically-based, robust, and provides a range of monitoring options depending on budget.

The following sections of the report are structured as:

- Methods and Data Analysis sections that combine both RB and EMB, as the methods and statistical analyses used were essentially the same for both regions;
- Separate results sections for each of RB and EMB detailing Spatio-temporal Variability within each region. These include univariate and multivariate analyses examining spatial variability within each region in 2016, and temporal variability among 2016 and earlier sampling years;
- Differences in faunal assemblages between RB and EMB, based on univariate and multivariate analyse of 2016 data;
- Taxonomic levels and data transformation section that combines RB and EMB;
- Shorebird prey biomass distribution section that combines RB and EMB;
- Monitoring Program for RB and EMB.

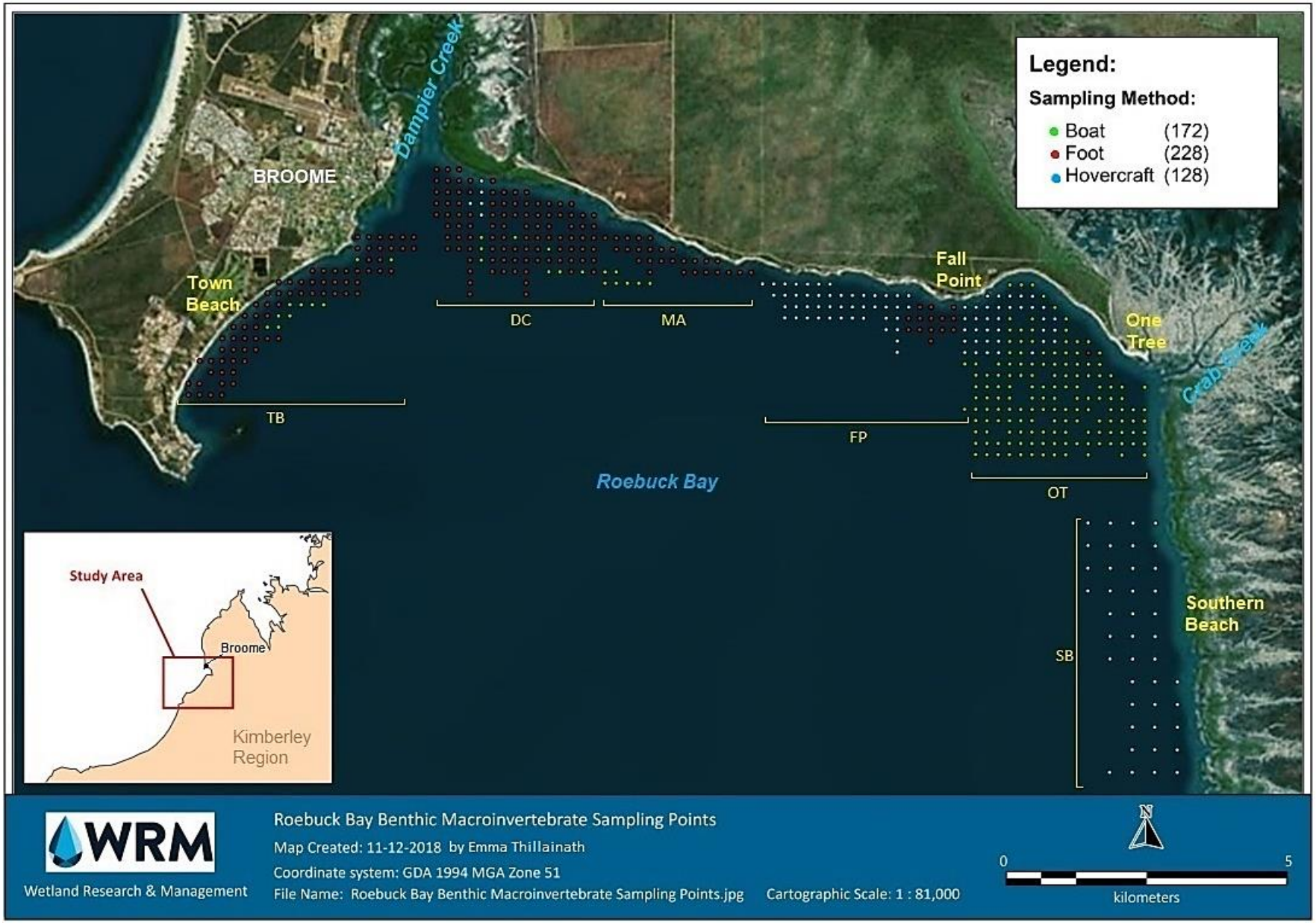


Figure 1. Roebuck Bay: location of stations most recently sampled in 2016. *A priori* areas used in the current report are also indicated (TB, DC, MA, FP, OT and SB; refer section 2.2).

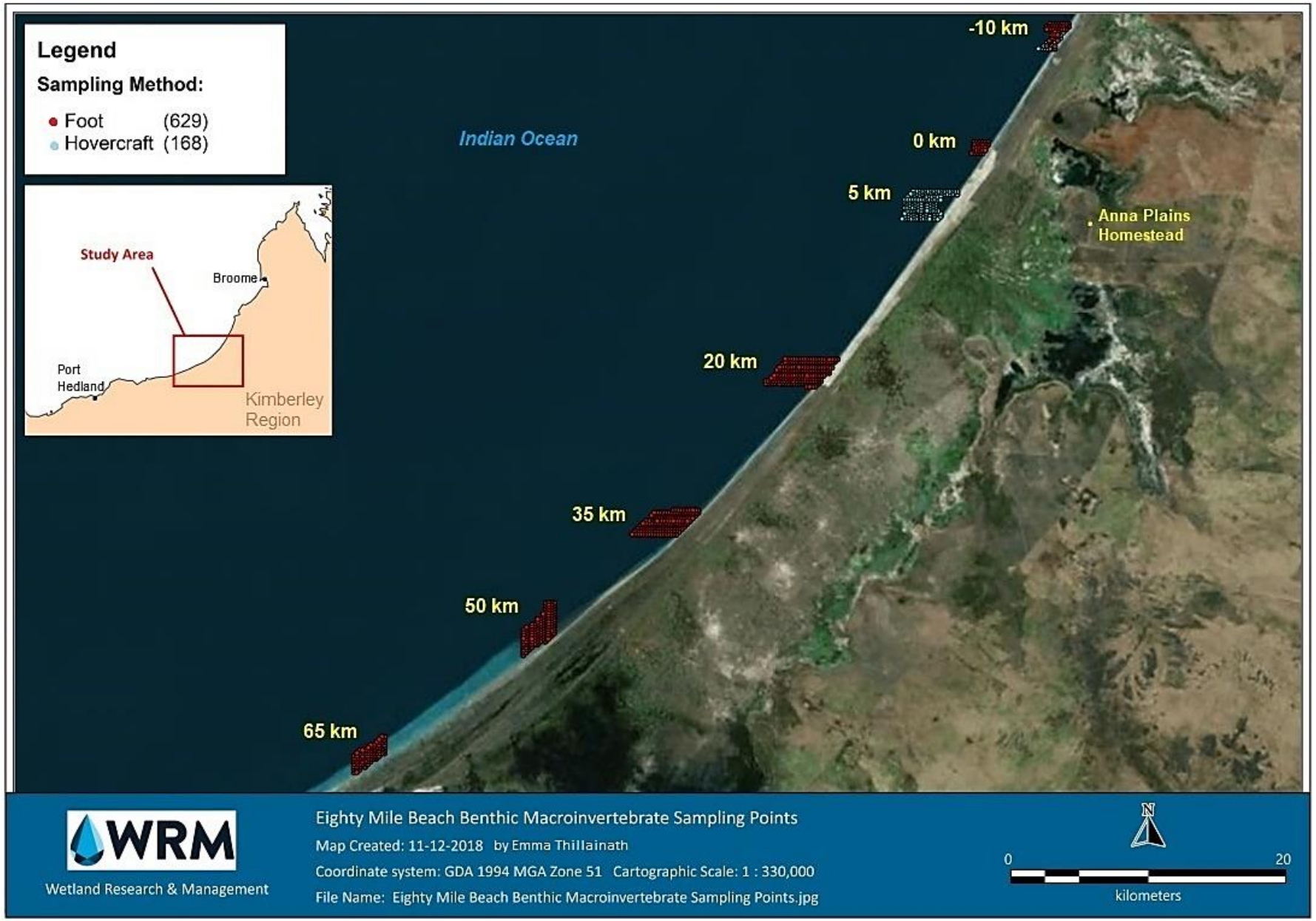


Figure 2. Eighty Mile Beach: location of areas and stations sampled in October 2016.

2.0 METHODS

2.1 Benthic Invertebrate Sampling

Detailed descriptions of sampling design and sampling stations for RB and EMB are provided in the AnnRoeBIM-16 *Field Report* (Piersma *et al.* 2016) and recent NIOZ report (Compton 2017). A summary of sampling methods for 2016 is provided in sections 2.1.1 and 2.1.2 below.

2.1.1 AnnRoeBIM-16

The **Ann**a Plains and **Roe**buck **B**ay **I**nvertebrate **M**apping program (AnnRoeBIM) commenced in June 1997 (RoeBIM-97; Pepping *et al*. 1999), with the first large-scale mapping expedition to RB. Since then, there have been four mapping expeditions to RB: March/April 2000 (Tracking-2000; Rogers *et al.* 2000), June 2002 (SRoeBIM-02; Piersma *et al*. 2002), June 2006 (RoeBIM-06; Drent *et al*. 2006) and current, October 2016 (AnnRoeBIM-16; Piersma *et al.* 2016). Sampling on Anna Plains (EMB) commenced in 1999 (AnnaBIM-99; Piersma *et al.* 2005) and there has been one expedition since; October 2016 (AnnRoeBIM-16; Compton 2017). These various expeditions to RB and EMB are collectively referred to as BIMs. Expeditions are timed to coincide with the austral spring when migratory shorebirds are returning from northern breeding grounds (August-October) and to avoid the wet season (November-February) when high air temperatures and rainfall make sampling and access difficult.

Historically, up to 537 stations in RB and 945 in EMB have been sampled, but not all stations have been sampled in all years (Figure 3A-B). Stations are laid out on tidal flats in a randomised systematic grid with 200 m intersections, but with distance of 400 m in the southeast of RB. Each station has a designated UTM co-ordinate (MGA51 datum) and a unique identifier based on easting and northing used to map benthic invertebrate abundance. Most of the RB system has been surveyed over the course of sampling (Figure 3A), however, because of its length, it has not been possible to survey the whole of the EMB system. Instead, seven gridded 'blocks' have been targeted, each block 15 km apart over ca. 75 km along the length of EMB (from 10 km north of the Anna Plains Station beach access, to 65 km south) (Figure 3B).

In October 2016, the AnnRoeBIM-16 program included 534 sampling stations in RB, and 816 stations in EMB (Figure 3A-B). As in previous years, sampling was typically conducted on foot using a PVC corer 10 cm in diameter and 20 cm in length (0.0083 m^2). At each station, three replicate cores were taken and bulked, with total surface area of 0.025 m^2. In areas difficult to access by foot, a boat was used, and three replicate cores taken using a 2 m long aluminium corer with a combined area of 0.025 m^2 (Piersma *et al*. 2016). A hovercraft was also used in RB to access sites in areas of deep/soft mud, with the same corer used as per foot sampling. Each bulk sample was sieved in the field using a 1 mm mesh sieve. Sieved samples were live-sorted in the laboratory at the Broome Bird Observatory (RB) or Anna Plains Station (EMB), and fauna identified and enumerated generally on the day of collection, or the following morning, by taxonomists attending the expedition. Fauna were identified to species wherever possible, but given the high diversity of the fauna and practicalities of time and budgets (in all years, not just 2016), morphotype was frequently used, based on the standardised nomenclature developed for RB and EMB. For simplicity, the term 'species' is used throughout this report to refer to the lowest taxonomic level achieved, but is synonymous with Operational Taxonomic Unit (OTU) of Compton *et al*. (2017). 'Species' is used here in preference to OTU, to distinguish this taxonomic level from higher order groupings at Family-Order and Class-Phyla levels.

Qualitative measurements of habitat characteristics were also made at each station, and these data were incorporated into analyses for the current report. Habitat characteristics included sediment penetrability (depth in cm of footprints made by samplers), seagrass and algal cover, and the presence of larger fauna on the surface (e.g. sentinel carbs, anemones, gastropods *etc*.) (Piersma *et al*. 2016). Penetrability is used as a relative measure to differentiate areas of firm sand from those of shallow, or deep soft mud.

Inundation time is also considered to influence benthic fauna distributions on tropical tidal flats, though tropical communities have been less well studied than temperate communities (Dittmann 2000, Mariano & Barros 2015). Carew and Hickey (2000) previously calculated inundation time for RB and EMB stations, based on tide tables and interpolation equations for mean tidal height, and expressed as percentage of time the water covers the tidal flat over the full tidal cycle (spring to neap tide). Elevations below mean spring low tide were defined as 100% inundation, while those above mean spring high tide were defined as 0% inundation (Carew & Hickey 2000).

Sediment samples have previously been collected from RB (1997, 2000, 2002, 2006) to quantitatively determine silt (< 63 µm diam.) content and median grain size (mgs), and to assess the relationship between benthic infauna and sediment characteristics. However different methods were used to analyse grain size pre- and post-2002; wet sieving in 1997 and 2000, and a particle size analyser in 2002 and 2006. While values for sediment grain size data are comparable between the two methods, silt values are not (see Compton *et al*. 2008 and references therein). In addition, not all sites were sampled and processed for sediments in all surveys, giving uneven coverage of the systems in each survey which limits ability to make direct comparisons. All sediment analyses were conducted by NIOZ, who also analysed sediment samples collected from EMB in 1999, using wet sieving. Detailed methods and results for sediment samples collected are not reported here but can be found in Compton (2017).

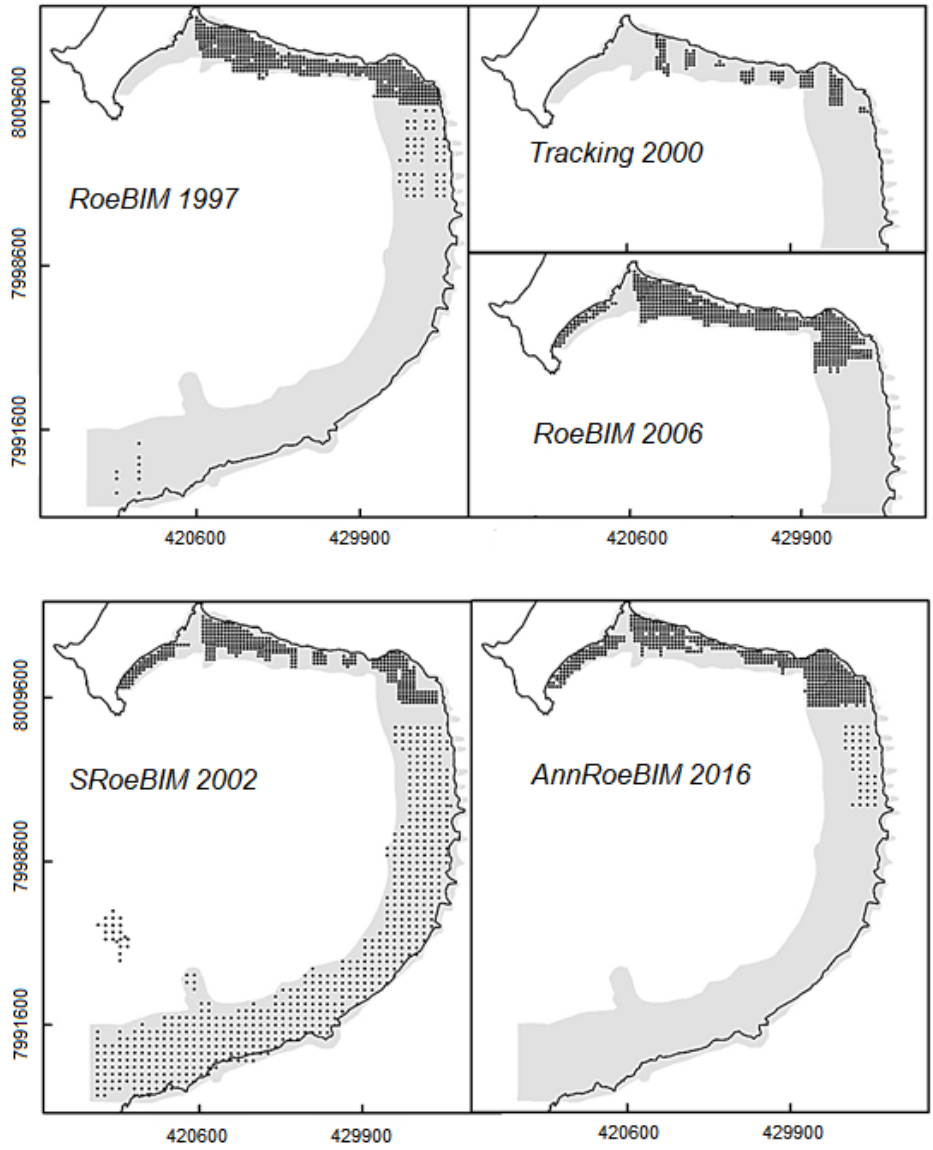


Figure 3a. AnnRoeBIM stations sampled at RBB in 2016 in comparison to the previous sampling in 1997, 2000, 2002 and 2006 (source: Compton *et al*. 2017).

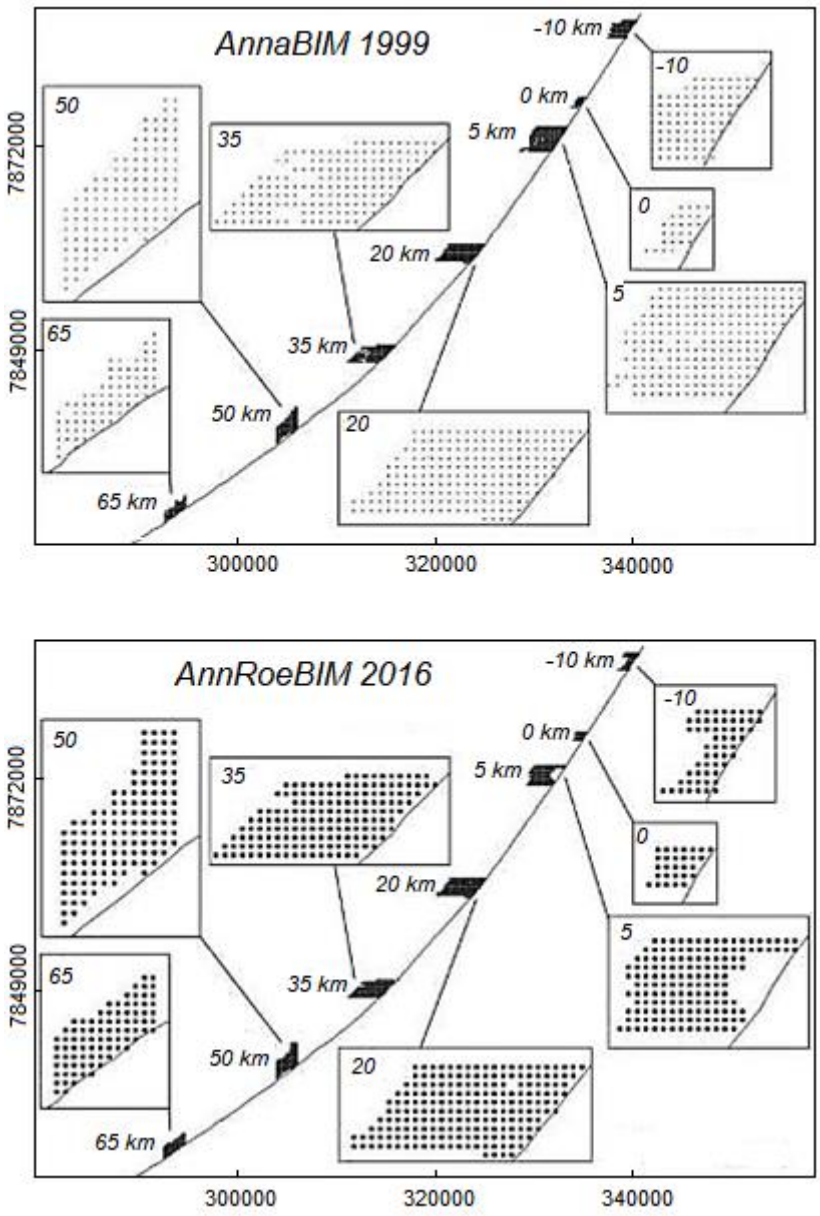


Figure 3b. AnnRoeBIM stations sampled at EMB in 2016, in comparison to the previous sampling in 1999 (source: Piersma *et al*. 2016). The seven sampling areas are coded by distance (km) from Anna Plains Station.

2.1.2 MonRoeb-16

The **M**onitoring **Roe**buck Bay **B**enthos (MonRoeb) monthly sampling program for RB commenced in 1996 with the aim of examining seasonality in benthic infauna (Compton 2017). The program involves monthly sampling on foot at two sites, 3 km apart, in Roebuck Bay; Falls Point (FP) and One Tree (OT) (Figure 4). Although intended to be monthly, sampling was most frequently conducted during the dry season months, as access is often problematic over the wet season (Compton 2017). At each site, two stations approximately 150 m apart have historically been sampled (inner station A and outer station B), with four replicate samples collected from each station. However, since 2003, station B at OT (OT-B) has not been sampled due to access difficulties (deep mud). Instead, this station was moved towards the shore, to station A (OT-A), and eight replicate samples collected. For all stations, each replicate sample consisted of a composite of six sediment cores (each 0.008 m^2) with a combined area of 0.048 m^2 (*cf* 3 cores per site for AnnRoeBIM). FP-A, FP-B and OT-A were again sampled in October 2016 using the same method as in previous years to allow direct comparisons with AnnRoeBIM-16 data.

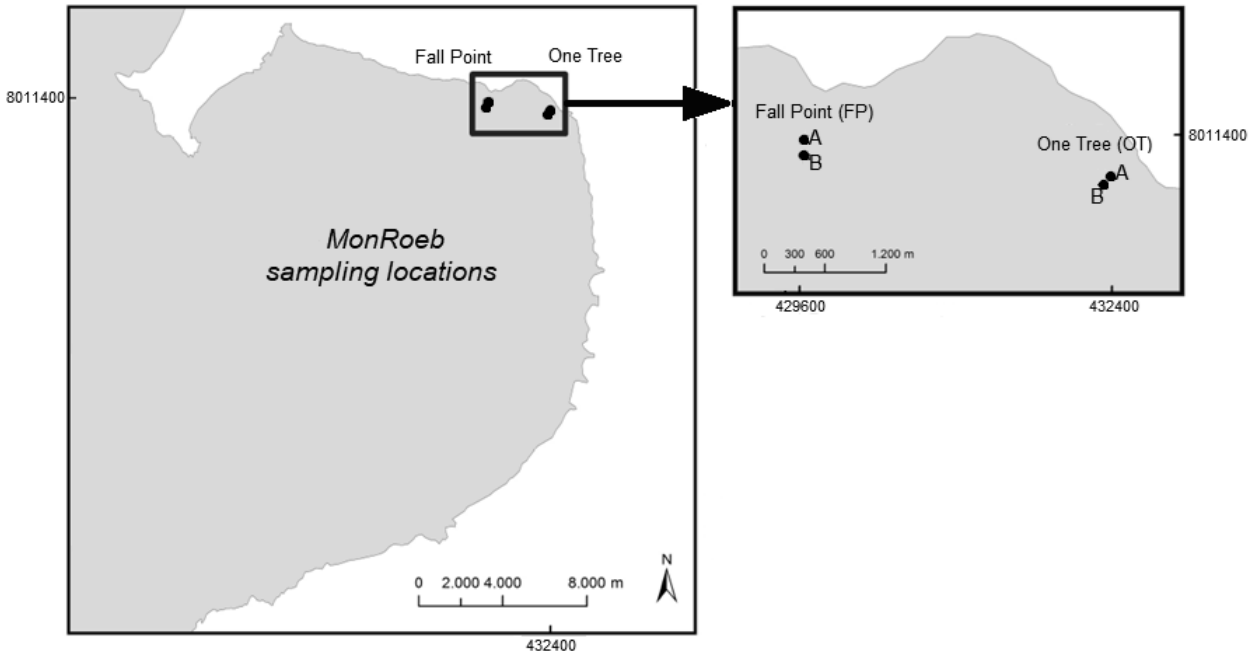


Figure 4. MonRoeb sampling sites (Fall Point and One Tree) and stations (A and B) in Roebuck Bay (source: Compton 2017).

Each bulk sample was sieved in the field using a 1 mm mesh sieve. Sieved samples were live-sorted in the laboratory at the Broome Bird Observatory, and fauna preserved in 4% formaldehyde for further taxonomic identification and enumeration. Fauna were identified to species wherever possible, or morphospecies using the standardised nomenclature applied to BIMs fauna (see section 2.1.1). Of note is the fact that from 1999 to 2005 inclusive, not all polychaetes were counted or identified, but only the families of the tubeworms Oweniidae and Chaetopteridae. From 2006 to 2016, all polychaetes were counted and where possible, identified to family level.

Qualitative measurements of key habitat characteristics were also made at each station. These included sediment penetrability (depth in cm of footprints made by samplers), seagrass and algal cover, and the presence of larger fauna on the surface (*e.g*. sentinel carbs, anemones, gastropods *etc*.) (Piersma *et al.* 2017). Sediments samples were also collected in 2016 (and 2002) to quantitatively determine median grain size and silt content, and to assess the relationship with quantitative penetrability measurements. At the time of writing, only the 2002 samples had been analysed for grain size (see Compton 2017).

2.2 Data Analysis

All 2016 data were entered into the AnnRoeBIM and MonRoeb MS Access databases developed as a collaborative project by NIOZ and DBCA and maintained by NIOZ and DBCA. The databases contain all biotic and abiotic information collected during macroinvertebrate mapping surveys of RB and EMB from 1997 to 2016. These databases were accessed for the current report and data exported in Excel format for further manipulation and statistical analysis.

2.2.1 Univariate Analyses

2.2.1.1 Pooling Samples

Frequency histograms for species richness and species accumulation curves for each area were used for initial exploratory examination of the AnnRoeBIM-16 data. Species accumulation curves were based on observed richness (Sob) and a range of commonly used richness estimators (Chao 1, Chao 2, Jacknife 1, Jacknife 2, Bootstrap, MM) which were generated using PRIMER v7 (Clarke & Gorley 2015). The initial investigations demonstrated that for a system with high overall diversity (RB = 322 species; EMB = 153 species), each sample typically contained low numbers of species per sample (*i.e.* RB = average of 8.1 taxa per sample, and EMB = average of 5.9 taxa per sample) but with high variability, which was not representative of a system with inherently high diversity. Subsequent exhaustive examination and discussion with peers (Marti Anderson, Massey University, NZ) substantiated the conclusion that the BIMs mapping programs were under-sampling taxa richness, at the sample level, especially given the high overall diversity for each system. Therefore, to reduce the variance in the data for meaningful statistical tests (Taylor 1961, 1971, Andrew & Mapstone 1987, Anderson & Santana-Garcon 2015), data from individual AnnRoeBIM-16 samples were pooled, *i.e.* abundances were summed for each species. Samples were pooled to avoid as best as possible the confounding effects of inundation and penetrability zones (see Results sections 3.2 and 5.2 for further discussion on Optimal Sampling Size). After additional investigation and substantial testing, the optimum for pooled samples comprised 9 cores, being data from 3 laterally adjacent sampling stations, with each sampling station consisting of 3 cores. Pooled stations were grouped by *a priori* area and sub-area as described below.

For RB, samples were first grouped into five *a priori* areas representative of broadly different geographical locations on a continuum around the bay from west to east; Town Beach (TB), Dampier Creek (DC), Middle Area (MA), Fall Point (FP), One Tree (OT) and Southern Beach (SB). Starting with the most south-western sample collected from Town Beach in 2016, all samples along the first UTM easting were then coded as 'A', samples along the second easting as 'B' and samples along the third easting as 'C'. This set of three sub-groups (A, B and C) was then classified as sub-area 1 (Figure 5). This was repeated sequentially for all eastings, and each set of three sub-groups (A, B, C) classified into sub-areas from 1 to 34 (Figure 5). For each sub-area, samples were then pooled (summed) by UTM northing, but only where there was a complete set of A, B and C samples for that northing. Samples from incomplete sets (*i.e.* 1 or 2 stations) were omitted.

The same approach was used for the seven areas sampled at EMB, except that sub-groups (A, B, C) and sub-areas (1 to 30) were determined by northing, rather than easting, given the orientation of EMB (Figure 6). Within each EMB sub-area, samples were then pooled (summed) by easting, rather than northing (Figure 6), but to again give composite samples of 9 cores across 3 stations.

Corresponding habitat data were averaged instead of summed, but otherwise treated in the same manner as species data for pooling.

All subsequent analyses of spatial and temporal variability using AnnRoeBIM data were conducted on pooled samples, with taxa richness and abundance summed across samples and environmental data averaged across samples.

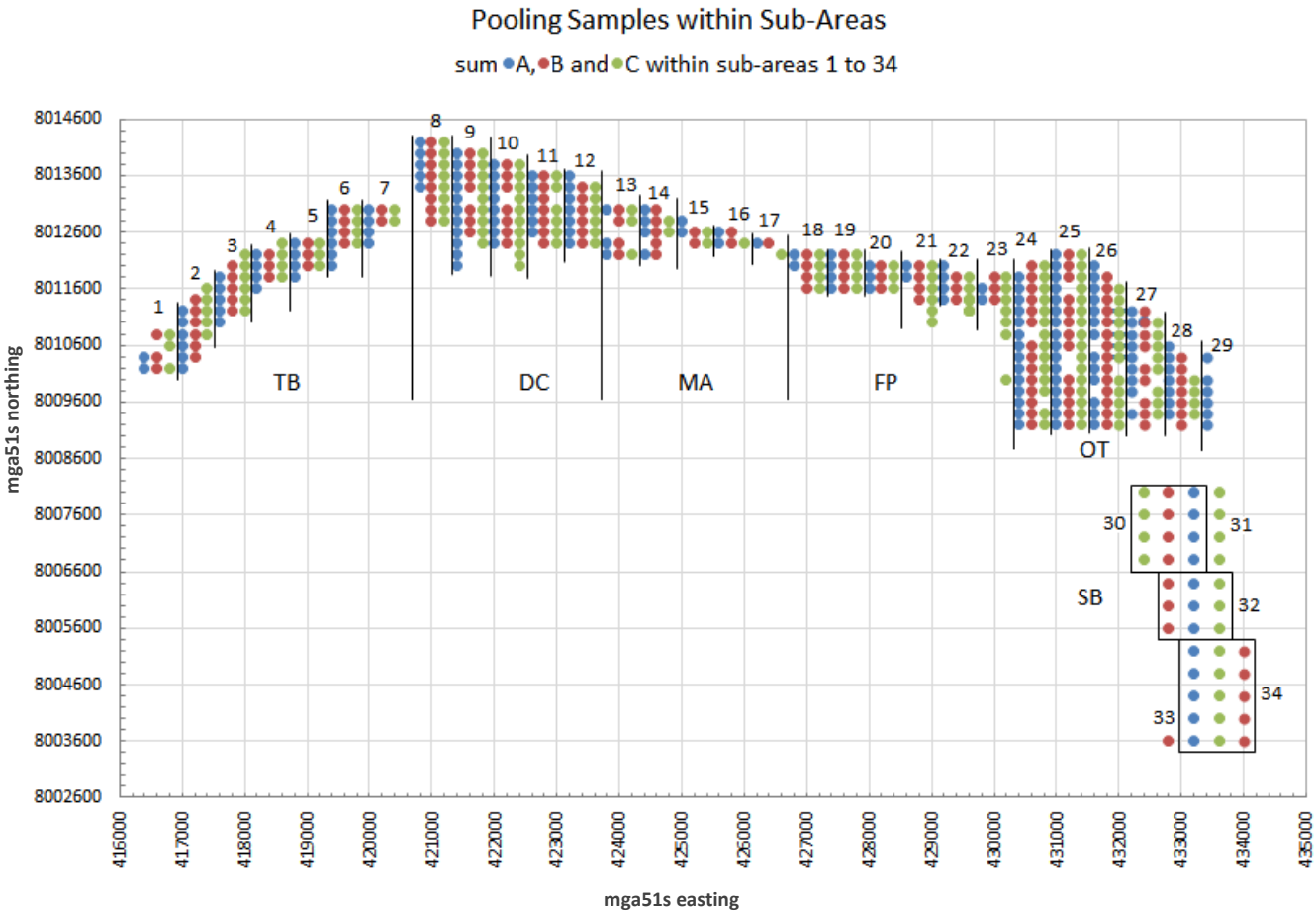


Figure 5. RB 2016: *a priori* sub-groups (A, B, C) and sub-areas (1 to 34) used to pool samples within six *a priori* areas: Town Beach (TB), Dampier Creek (DC), Middle Area (MA), Fall Point (FP), One Tree (OT) and Southern Beach (SB).

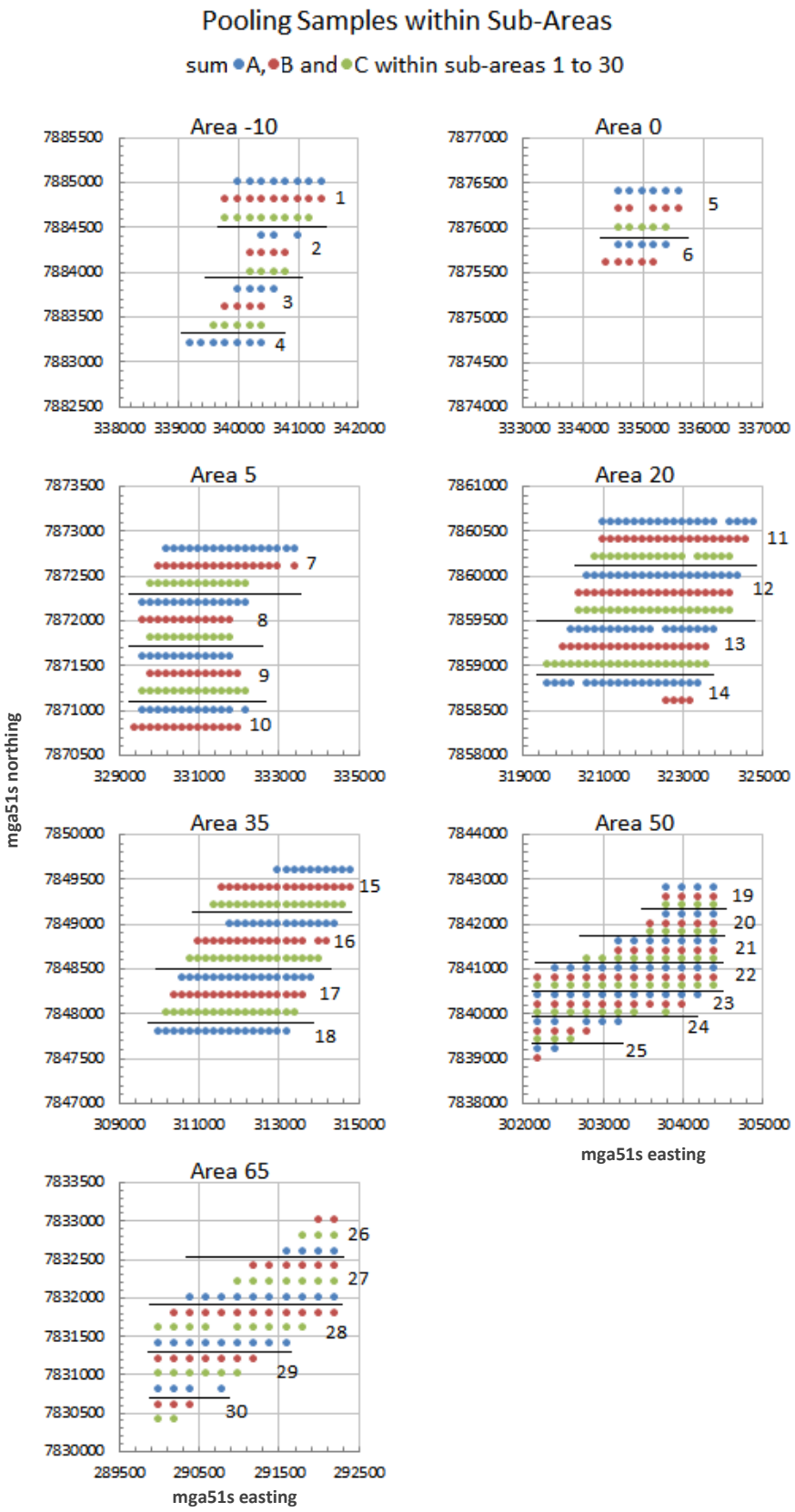
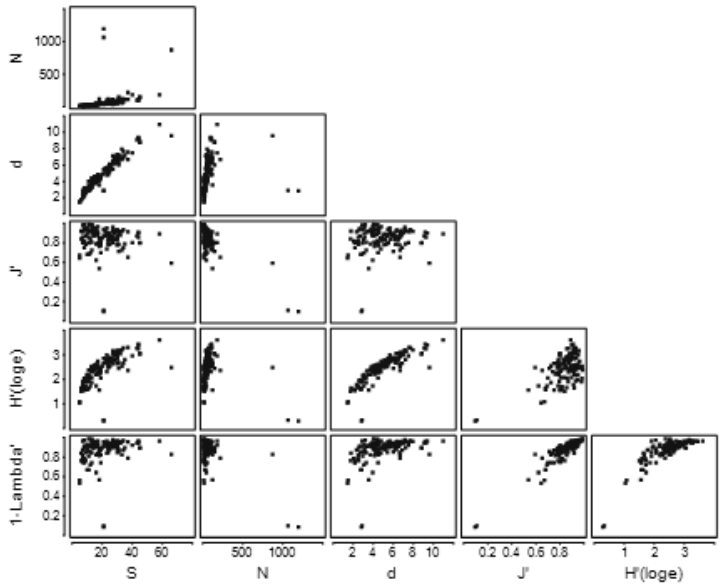


Figure 6. EMB 2016: *a priori* sub-groups (A, B, C) and sub-areas (1 to 30) used to pool samples within the seven sampling areas: -10, 0, 5, 20, 35, 50 and 65. Refer Figures 2 and 3b for location of sampling blocks along EMB.

2.2.1.2 Diversity Measures

In addition to the total number of species (S) and the total number of individuals (N) in each sample, several structural diversity indices were investigated as potential indicators for future monitoring. An enormous variety of structural diversity indices are described in the published literature as sensitive to ongoing monitoring, so for the current study a sub-set was chosen from those most frequently used and those considered most applicable to marine intertidal benthic invertebrate populations. These included conventional measures of richness and evenness, namely Shannon-Wiener diversity (*H'* $\log_e$), Margalef's index (*d*), Pielou's evenness index (*J'*) and Simpson index (1-λ'). Relationships amongst these measures were investigated using Spearman rank correlation which revealed strong positive relationships between *S* and *d* ($r^2 > 0.83$, $p < 0.01$), and between *J'* and 1-λ' ($r^2 > 0.92$, $p < 0.01$) based on the 2016 datasets for each of RB and EMB (Figure 7). Consequently, only S, N, *J'* and *H'*($\log_e$) were considered for further analyses.

(a) RB 2016



(b) EMB 2016

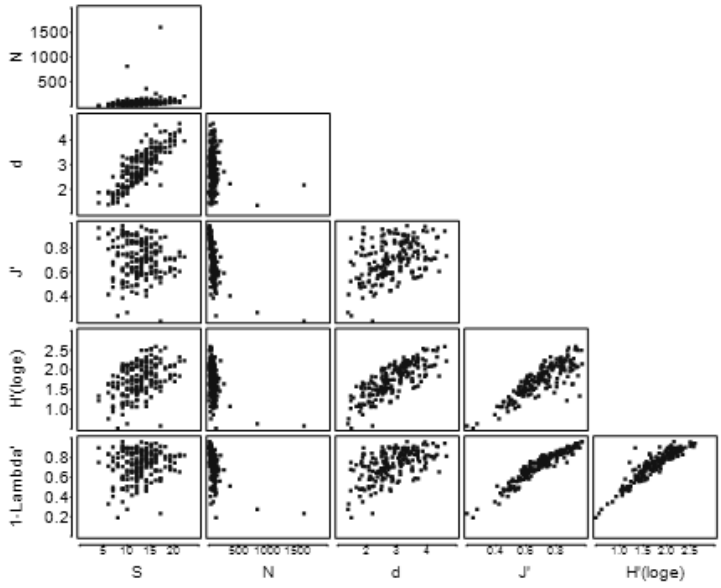


Figure 7. Relationships amongst species-level structural diversity measures for benthic invertebrate communities, based on pooled samples for each of (a) RB, and (b) EMB. Symbols for measures: *S* = total number of species; *N* = total number of individuals; *H'* $\log_e$ = Shannon-Wiener diversity, *d* = Margalef's index; *J'* = Pielou's evenness index; 1-lambda' = Simpson index.

2.2.1.3 Spatial and Temporal Variability

Species-habitat relationships were analysed using Spearman rank correlation (rho) on AnnRoeBIM-16 data for all areas combined and for individual areas within each of RB and EMB. Where significant correlations were found, stepwise multiple regression analysis was then applied to determine the relative strength of any linear relationships for species metrics (S, N, *J'* and *H'*($\log_e$)) with the various habitat variables. Relationships were visually checked using scatter plots. To reduce the number of different habitat types potentially required for on-going monitoring, qualitative categories for inundation and penetrability were assigned *a priori*, and together with quantitative data, subjected to correlation and regression analyses. Qualitative inundation categories included: 0-30%, 31-70% and 71-100%. Qualitative penetrability categories included: 0-10 cm, 11-20 cm, 21-30 cm, 31-40 cm and 41-50 cm. For RB, geographic location was also included as a habitat variable, by calculating the linear distance of each sampling station from an arbitrary start point to the west of Town Beach at Broome township.

One-factor analysis of covariance (ANCOVA) was then used to test for statistically significant differences in species metrics among areas and among sub-areas within each area, with inundation and penetrability as the covariates. For RB, areas and sub-areas were effectively used as factors of distance around the bay. Two-factor analysis of variance (ANOVA) was used to separately test for effects of seagrass or algal cover for those areas where these habitats occurred, and within which there were a sufficient number of replicate samples to test between zones with and without seagrass or algae (*i.e*. Area x Seagrass or Area x Algae).

Two-factor ANOVA was also used to test for significant year and sub-area effects (Year x Sub-Area) on species metrics within *a priori* areas, using combined historic and 2016 data for each of RB (1997, 2000, 2002, 2006, 2016) and EMB (1999, 2016).

Prior to all ANCOVA / ANOVA testing, the assumptions of normality and homogeneity of sample variances were checked using Shapiro-Wilk (Shapiro & Wilk 1965) and Levene's (Levene 1960) tests, respectively. Where these tests returned significant results, data were $\log_{10}(x+1)$ transformed to conform to the assumptions of the ANCOVA / ANOVA test. This was in acknowledgement that it was not always possible to meet the assumptions (even with severe transformation such as log) and non-parametric alternatives would have lower statistical power to detect change, given the nature of the data.

Unless stated otherwise, all univariate statistics were performed using IBM SPSS Statistics (v22.0).

2.2.1.4 Detectable Change

Power analysis (R 3.4, R Core Team 2017) was used to calculate sample size for varying effect sizes; *i.e*. number of samples needed to detect 10%, 20%, 30%, 40% or 50% decline in species richness and abundance. Analyses were based on a one-sample, one-tailed t-test, and were performed on raw MonRoeb-16 data for stations, as the MonRoeb data provided a larger number of replicate samples for single locations than the AnnRoeBIM data, *i.e*. 6 cores per sample per station, with 4 samples for of FP-A and FP-B, and 8 samples for OT-A. In addition, these data were not spatially confounded as could occur by pooling the AnnaRoeBIM samples to improve taxa richness. Power analysis was conducted by taking the average (mean) and standard deviation (SD) for each area, progressively reducing the average to simulate a decline (*i.e*. effect size) and assessing changes in replication needed to achieve adequate power (power = 80%, α = 0.05).

2.2.2 Multivariate Analyses

In addition to univariate species metrics, the multivariate parameter of Bray-Curtis similarity in species assemblage composition was also examined as a potential indicator for monitoring. In contrast to univariate analyses, which look at differences in individual descriptors of assemblage composition (*i.e.* richness, diversity *etc.*), multivariate analyses can capture differences in whole assemblage composition. For example, two successive samples from a site may have the same richness, diversity, abundance and biomass, but have totally different assemblage composition, and univariate methods will not detect this difference, whereas multivariate approaches will.

2.2.2.1 Pooling Samples

In the first instance, shade plots based on Bray-Curtis dissimilarity/similarity measure (Clarke *et al.* 2014) were used to visually examine the effectiveness (or otherwise) of pooling on species assemblage data. The plots were generated using PRIMER v7 (Clark & Gorley 2015). Shade plots were also used to decide on the best data transformation (*i.e.* square root, fourth root, log, presence-absence) for multivariate analyses to avoid the dissimilarity/similarity calculation being dominated by just a couple of species with occasionally large abundance. In this case, fourth root transformation appeared the best compromise between no transformation and log, the latter being similar to presence-absence in terms of loss of information.

The method described by Anderson and Santana-Garcon (2015) for choosing the number of samples to pool for multivariate analysis of community data was also applied. This method randomly pools samples in order to generate several different series of data, each series comprised of a different number of pooled samples. The dissimilarity (d; in this case Bray-Curtis) within each series was then compared by plotting the mean (with 0.025 and 0.975 quantiles) proportion of dissimilarities of 100%, *i.e*. d = 1 (no species in common) as well as the mean proportion of undefined dissimilarities, *i.e.* d = “not a number” (NaN), caused by samples with zero species. The minimum number of pooled samples that reduced both these proportions to zero, or close to zero, was considered the appropriate number of samples to pool. These analyses were performed in R 3.4 (R Core Team 2017) using the R code provided in Anderson and Santana-Garcon (2015).

Preliminary analyses using Bray-Curtis resemblance matrices and non-metric Multi-Dimensional Scaling (nMDS) ordination performed by PRIMER v7 (Clark & Gorley 2015), showed that even after pooling, many samples had no species in common, and the stress for the two-dimensional (2D) nMDS ordination plots was is too high to interpret with certainty. This was particularly the case for the RB data. However, the 2D ordination plots were interpretable to the extent that they showed similar patterns to the three-dimensional (3D) solution, and the latter had acceptable stress.

2.2.2.2 Spatial and Temporal Variability

The k-R clustering (non-hierarchical) procedure, together with similarity profile analysis (SIMPROF) in PRIMER v7 was used for an exploratory search for natural groupings of samples within the (likely) graduation of change in species assemblages around RB and along EMB (Clarke *et al.* 2008). k-R clustering is a non-parametric analogue of k-means clustering. The number of possible cluster groups (*k*) was arbitrarily limited to four for RB and five for EMB, as this offered more plausible (and readily interpretable) structures in regard to likely influencing habitat variables, *i.e*. inundation, sediment grain size and geographical location. k-R clustering was also used to investigate similarities/dissimilarities between MonRoeb16 and AnnRoeBIM-16 datasets (*k* = 5 groups).

Multivariate relationships between species assemblages and the habitat variables were analysed using both BIO-ENV (based on Spearman rank correlation; Clarke & Warwick 2001) and nonparametric multivariate multiple regression (DistLM) add-on to PRIMER v7 (McArdle & Anderson 2001, Anderson 2001, 2002). BIO-ENV and DistLM were performed on AnnRoeBIM-16 data for each of RB and EMB using all pooled samples. BIO-ENV defines the suite of environmental variables best correlated with fauna assemblages based on rank correlation between the similarity matrix generated for the fauna data and matrices generated for all combinations of environmental data. The square of the Spearman rho value, output from BIO-ENV, represents the proportion of shared variance between the fauna and environmental datasets. DistLM similarly tests for significant environmental variables that explain the observed similarity/dissimilarity among fauna assemblages, based on multivariate linear regression. The final multivariate multiple regression model that best explained the variation in the species data was chosen using the stepwise forward-selection procedure and Akaike information criterion (AIC). AIC is one of several options available within the DistLM program, to help select the most parsimonious suite of environmental variables with the best linear relationship to the species assemblage data. Significance of regression relationships was assessed using 9999 random permutations of the data. Distance-based redundancy analysis (dbRDA) plots were constructed to visualise the relative importance of the habitat variables to patterns in species assemblage data. These analyses were performed based on the correlation of Bray-Curtis dissimilarity matrices for species abundance (fourth root transformed) with Euclidean distance matrices of habitat data (log_{10} transformed where necessary).

The permutational multivariate analysis of variance (PERMANOVA) add-on to PRIMER v7 was used to test for significant differences in species assemblages among *a priori* areas, sub-areas and years (Anderson 2001a,b, McArdle & Anderson 2001, Anderson *et al.* 2008). One-factor PERMANOVA using AnnRoeBIM-16 species abundance data (fourth root transformed) was used to test for significant differences between/within areas including habitat variables identified from DistLM as covariates. Two factor PERMANOVA was used to separately test for effects of seagrass or algal cover for those areas where these habitats occurred, and within which there were a sufficient number of replicate samples to test between/within zones with and without seagrass or algae (Area x Seagrass or Area x Algae). Two-factor PERMANOVA was also used to test for significant year and sub-area effects (Year x Sub-Area) on species metrics within *a priori* areas, using combined historic and 2016 data for each of RB and EMB.

Assumption of homogeneity of dispersion (heteroscedasticity; PERMDISP, $p > 0.05$) was satisfied for comparisons between most RB areas, except between TB and MA; therefore, PERMANOVA results comparing between these areas, should be viewed with caution. Similarly, with the exception of results for MA and FP, results for comparisons among years, should be viewed with caution.

Distances among centroids was used to examine the relative positions of *a priori* area and sub-area groups in ordination space (analogous to means plot for univariate data) (see Anderson 2017). Threshold metric MDS (mMDS) ordination was performed on the dissimilarity/similarity matrix generated for the centroids, and the resultant ordination plot used to visualise the patterns. mMDS rather than the more typical non-metric MDS (nMDS) was used, as the former better preserves the original dissimilarity scale (in this case Bray-Curtis).

3.0 SPATIO-TEMPORAL VARIABILITY WITHIN ROEBUCK BAY

3.1 Dominant Environmental Factors

Community structure in marine intertidal mudflats is generally considered to be influenced by both species interactions and habitat variability, the latter determined by large-scale processes such as tidal cycle (inundation time), sand and silt transport, and, as well as small-scale processes such as sediment type (grain size) and vegetation cover (Alongi 1987a-c, Lana & Guiss 1992, Snelgrove & Buman 1994, Rodrigues *et al*. 2006, van der Heide *et al*. 2012). Several studies, mostly in temperate regions, have suggested there are particularly strong relationships between the structure of the intertidal benthic assemblages and grain size. For instance, Lu *et al*. (2008) observed a positive correlation between the fine fractions (silt and clay) and richness and diversity. Thrush *et al*. (2003) used mud to predict macrofaunal species occurrence along an estuarine gradient. However, the relative extent to which large- and small scale-processes effect distribution and abundance of species in tropical intertidal mudflats has not been widely studied. This appears to be partly due to the typically low number of animals in individual samples and high variability in species among samples (see Dittmann 2000, 2002, Mariano & Barros 2015).

Inundation, penetrability and seagrass/algae/*Lyngbya* cover are the dominant physical variables measured as part of invertebrate mapping surveys for RB (see section 2.1.1), with inundation and penetrability identified as influential parameters in RB by Compton (2017). These were evaluated in the current analyses as potential predictors of benthic invertebrate assemblages.

Figure 8 illustrates the qualitative inundation categories used here for RB, though both qualitative categories and continuous data were input to analyses. Qualitative categories ranged from 0 - 30% close to the shore, 31 - 70% in mid zones, and 71 - 100% furthest out.

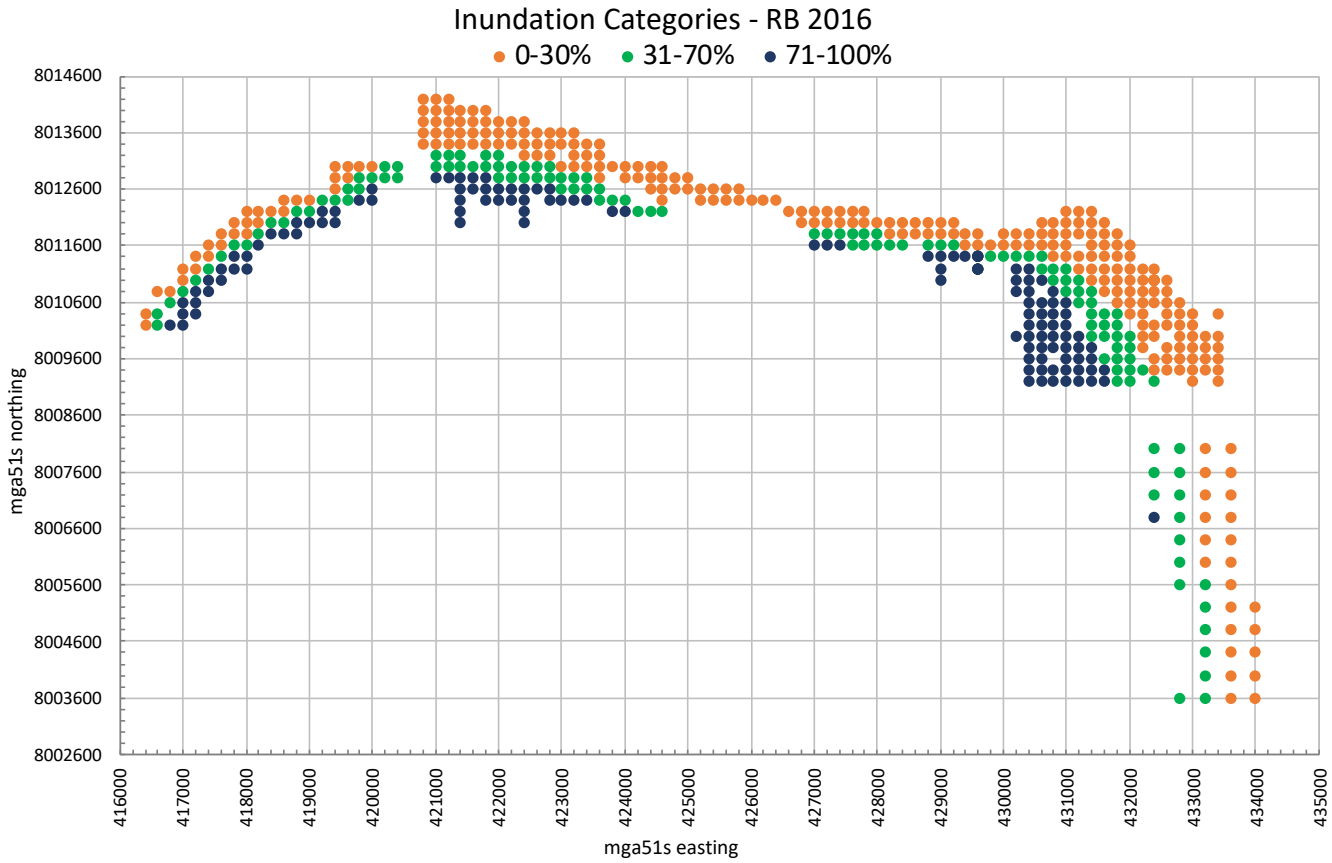


Figure 8. RB 2016: inundation category (0-30, 31-70, 71-100%) for each station sampled.

While calculated duration of inundation does not change, the number of stations sampled within each inundation zone varies from year-to-year dependent on the sampling design and ultimately budget of the specific expedition; not all expeditions could or intended to sample all stations in RB.

Penetrability has been measured in most years, but there are fewer data on sediment grain size and silt content. Correlation and regression analyses were therefore used to examine the relationships between penetrability in 2002 and 2006 and corresponding data for silt content and median grain size (mgs), to determine if penetrability was a suitable surrogate for silt content and grain size. Results for 2002 data showed a moderately-strong significant relationship between penetrability and both silt content and mgs, while the 2006 data showed similar but weak relationships (Figure 9). The fact that only weak relationships were recorded for 2006 was considered due to the much smaller sample size in 2006 (n = 125) compared to 2002 (n = 665), with the expectation that had more samples been collected, similarly strong relationships to that observed for 2002 would also have been recorded for 2006. Based on this, penetrability was considered a reasonable surrogate for use where data on silt content or grain size were absent, such as 2016.

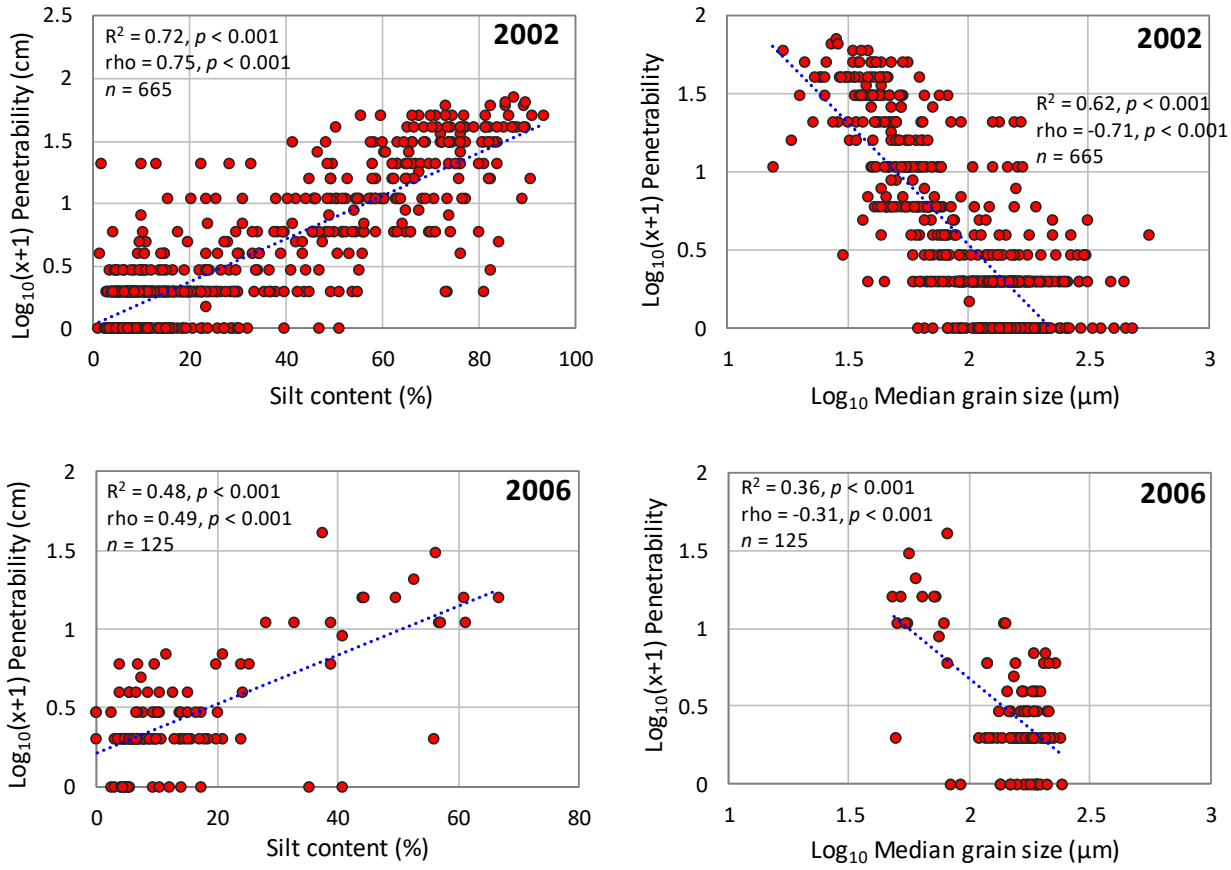


Figure 9. RB: relationship between sediment penetrability, percent silt content (< 63 µm) and median grain size, based on data for June 2002 and June 2006. Linear trend line, linear regression coefficient (R^2), Spearman rho and significance level are indicated for each plot.

Spatio-temporal variability in penetrability values between 2002 and 2016 is depicted in Figure 10. In general, deeper fine-grained muds, up to 50 cm penetrability, dominate the OT and SB areas, while relatively course sands of 0 - 10 cm penetrability tend to dominate in other areas. As noted by Piersma *et al.* (2016), this broad pattern did not appear to have changed significantly between 2002 and 2016 although people involved in all surveys noted there had been a redistribution of sediments over time,

especially within FP area in RB and in several of the more norther blocks at EMB. In 2016, OT was mostly sampled by boat, and therefore penetrability data were not recorded for many of the stations in this area.

Qualitative categories for sediment penetrability derived for 2016 data are illustrated in Figure 11. Again, for the current report, both qualitative category and continuous data were used to investigate spatial patterns in the benthic invertebrates.

Seagrass beds occur in the shallow sandy nearshore areas and intertidal mud flats of the bay, though their distribution and abundance can be highly variable year-to-year. Species include *Halodula uninervis* ("linear seagrass") and *Halophila ovalis* ("oval seagrass"), both of which were particularly abundant along Town Beach in 1997. Significant declines in both species of seagrass were recorded for the lower north shores due to Cyclone Rosita in April 2000 (Piersma *et al.* 2016). This was followed by strong recovery of *H. ovalis* in 2006, though distribution had shifted westward. By October 2016, both species had re-colonised parts of Town Beach, Dampier Creek and the Fall Point area (Piersma *et al*. 2016).

Blooms of the toxic cyanobacteria *Lyngbya majuscula* also occur in the bay, and there is concern that major blooms have caused significant changes in the intertidal benthic community composition, and affected shorebird foraging behaviour in the bay (Estrella 2013). It is not clear if these blooms are driven by diffuse source nutrient inputs from urban areas around Town Beach / Simpson Beach and Dampier Creek, or point source nutrient inputs from episodic sewage releases from the Broome South Wastewater Treatment Plant, or some other source (see McMahon & Dunham 2017).

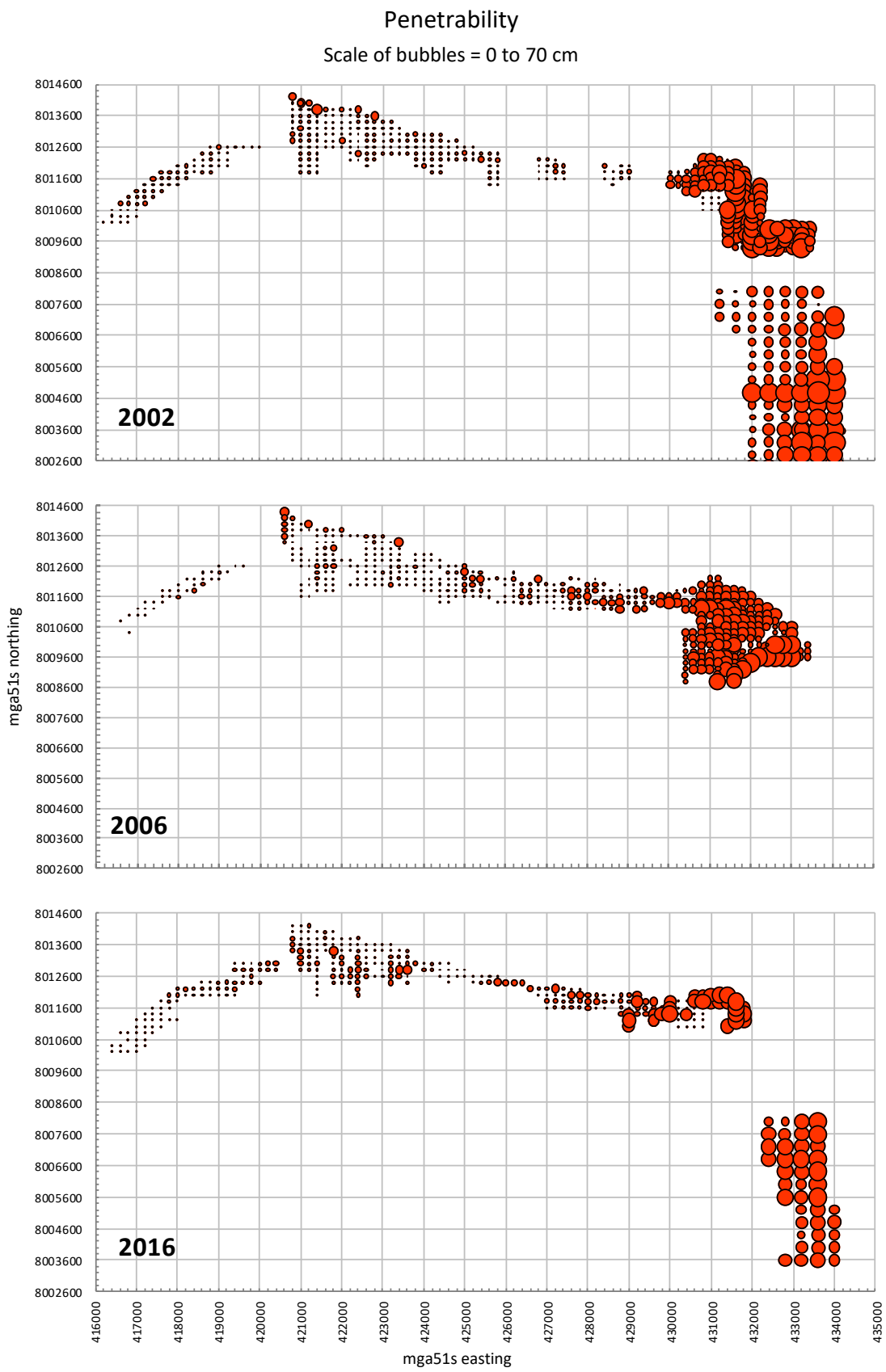


Figure 10. RB: Sediment penetrability in intertidal areas sampled in June 2002, June 2006 and October 2016.

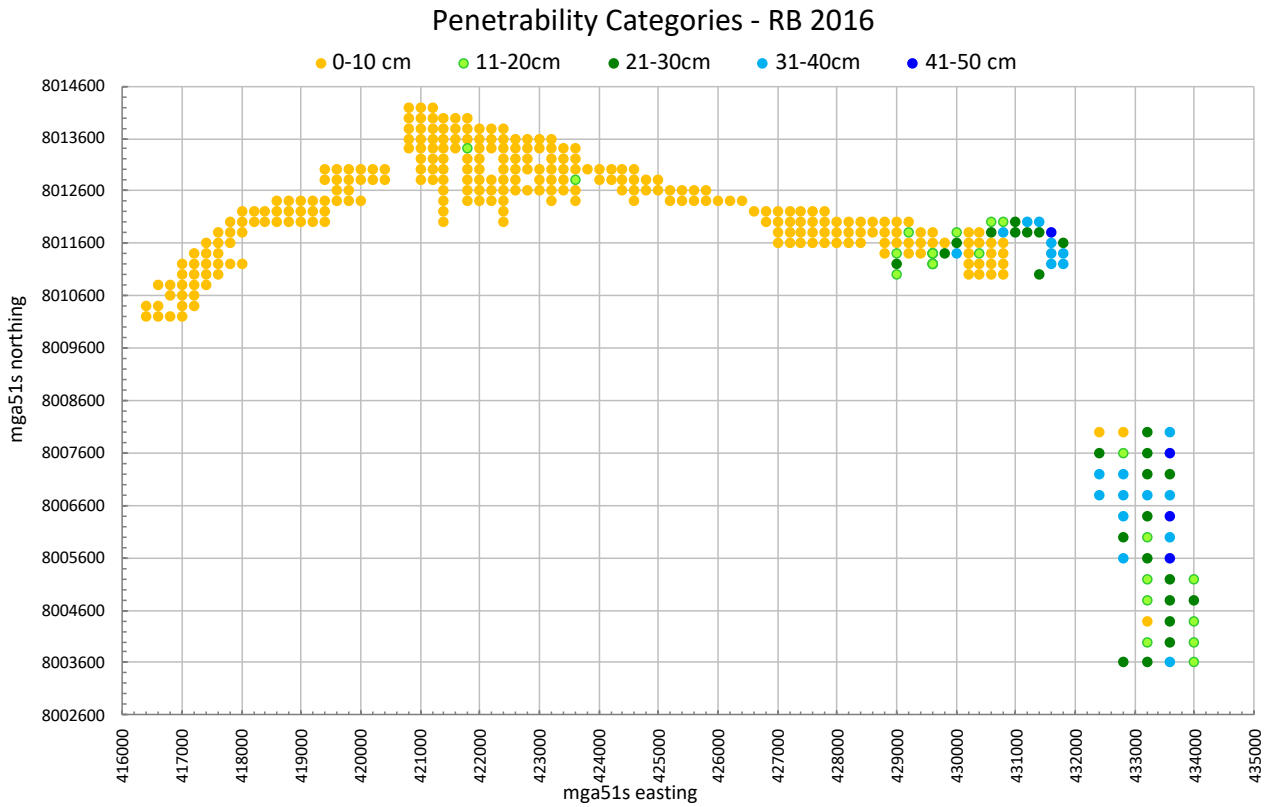


Figure 11. RB 2016: penetrability category (0-10, 11-20, 21-30, 31-40, 41- 50 cm) for each station.

3.2 Optimal Sampling Size (Number of Cores) for Benthic Invertebrates

Spatial distribution, or dispersion, of individuals in marine benthic invertebrate communities is usually contagious, that is, the presence of one individual increases the likelihood that one or more other individuals will occur close by (see Elliot 1977, Hughes 1984). Typically, there are patches of high density where individuals of one or several species occur in clumps, influenced by environmental factors and predator-prey relationships. Larger aggregations of clumps may also occur over larger spatial scales. Therefore, it is important to determine not only the quadrat size (sampling unit size), but also the mathematical model, that best fits the population, in order to accurately assess temporal and spatial changes, as well as the effect of environmental variables (see Underwood 1997, Anderson 1998, Leonardsson *et al*. 2016).

The combined AnnRoeBIM-16 and MonRoeb-16 sampling for Roebuck Bay recorded 12,565 benthic invertebrate specimens, representing 360 species from 22 phyla. Relative differences in the total number of species recorded for each station sampled are illustrated in Figure 12a.

For AnnRoeBIM-16, 328 species were recorded from the 534 samples, with an average of 8.1 species per sample, and a range in values of 0 - 35. Approximately 40% of samples (*i.e.* 213) recorded ≤ 5 species, while 4% (22) recorded no species. Bray-Curtis pairwise percent dissimilarity/similarity analysis on the 511 samples that contained species, showed that of the 130,560 pairwise combinations of samples, 55,678 pairs (*i.e*. 43%) had no species in common. This pronounced variability suggested the sampling size (3 cores/sample, total surface area = 0.025 m^2) was too small to be informative of the species assemblages present (Clarke *et al*. 2006).

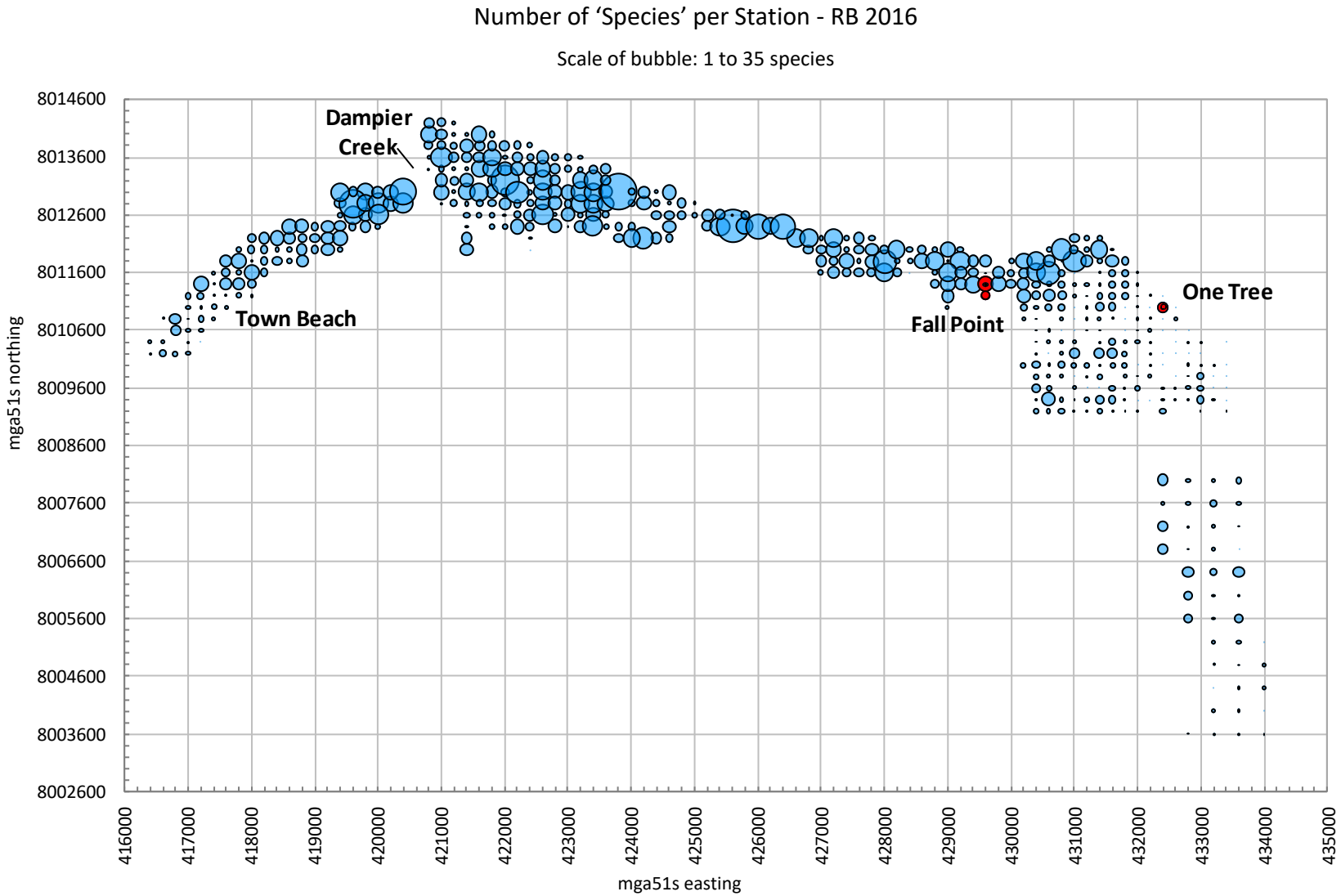


Figure 12a. RB: total number of benthic invertebrate taxa (species-level) recorded from each station sampled in October 2016, including AnnRoeBIM-16 and Monroeb16 (red) programs.

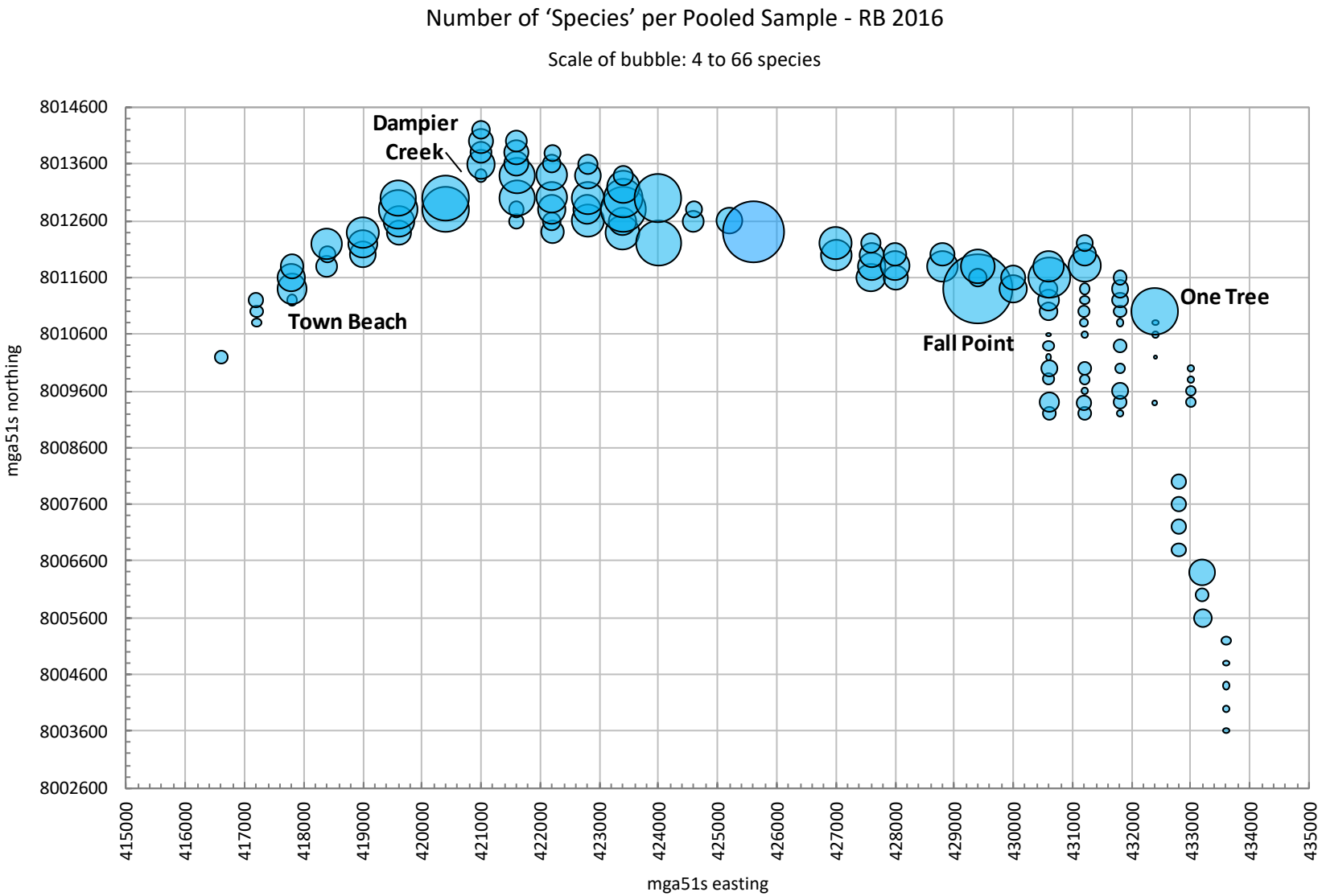


Figure 12b. RB: total number of benthic invertebrate taxa (species-level) calculated for each pooled sample for AnnRoeBIM-16.

Review of the published literature showed there is a wide array of sampling unit sizes for benthic intertidal fauna sampling, both for Australia and overseas. Studies on soft-sediment macrobenthos commonly use Perspex or PVC corers similar to those used for the current RRB and EMB surveys (Dittmann 2000, Ysebaert & Herman 2002, Anderson *et al.* 2004, Magni *et al*. 2006, Dolbeth *et al.* 2007, Mariano & Barros 2015, Checon *et al.* 2017, Hamylton & Barnes 2018, and other studies cited therein). The diameter (and length) of the corers and number of replicate samples per station varies dependent on the habitat type and heterogeneity, accessibility, aims of the study, expected statistical power for detecting change, and available budget. While the literature search was by no means exhaustive, most studies that employ univariate or multivariate analysis to statistically quantify spatio-temporal variability, appear to rely on a greater number of replicate samples per station than the 3 cores (combined surface area = 0.025 m^2/station) used for RB and EMB. Sampling designs also typically include multiple stations within distinct sub-areas determined by known or presumed spatial gradients in environmental parameters and/or biota.

For example, Dittmann (2000) used a stratified random sampling approach to study benthic communities in a tidal flat of the Haughton Estuary near Townsville, Queensland. Data from 15 samples collected on two occasions in 1991 (April and September) were analysed using both univariate and multivariate techniques. The samples were collected from each of 5 transect sites, each site 100 m^2. Each of the 15 samples comprised 5 replicate cores for each of three fauna groups; macrofauna, mesofauna and meiofauna. Macrofauna were sampled using a corer of 0.0177 m^2, (combined surface area = 0.088 m^2) mesofauna with a 0.001 m^2 corer (combined surface area = 0.005 m^2), and meiofauna with a 0.0005 m^2 syringe (combined surface area = 0.0025 m^2). The total combined surface area sampled was therefore ~0.1 m^2/site.

Ysebaert and Herman (2002) quantified spatio-temporal variability in benthic macrofauna in intertidal soft-sedimented habitats of the Schelde Estuary, The Netherlands. A hierarchical sampling design and canonical correspondence analysis was used to examine environmental predictor variables. Four intertidal locations were randomly selected, with a differing number (2 to 4) of sampling stations at each location, with a total of 30 stations sampled across all locations. Each station was sampled annually in autumn (September - October) between 1994 and 2000. At each station, 15 replicates were taken with a 0.024 m^2 corer (combined surface area = 0.36 m^2) plus 5 replicates with a 0.0884 m^2 corer (combined surface area = 0.44 m^2). The total combined surface area sampled was therefore ~0.8 m^2/site.

Anderson *et al*. (2004) used univariate and multivariate analyses to model relationships between benthic macrofauna and various environmental parameters in mudflats of Okura Estuary, New Zealand. Sampling was stratified by season, with each of 15 sites (each site 50 m x 25 m) sampled on each of 6 occasions between August 2001 to April 2002. At each site on each occasion, 6 random replicate samples were collected with a 0.033 m^2 corer, with a combined surface area of ~0.2 m^2/site.

Mariano & Barros (2015) investigated relationships between benthic macrofaunal assemblages and environmental variables in intertidal areas of three tropical estuaries in Baía de Todos os Santos, the second largest bay in Brazil. Multivariate analyse were used to evaluate similarities in macrofaunal assemblage structures, and relationships with environmental variables. Sampling was repeated on 3 occasions (March, June and October 2011) at 10 - 11 stations in each of 3 soft-bottom intertidal areas. At each station, 8 random replicate samples were collected using a 0.0177 m^2 corer, with a combined surface area of ~0.14 m^2/site.

The total surface area sampled per station for RB and EMB (0.025 m^2) would thus appear to be less than 10% of that sampled for other similar studies of benthic macrofauna in soft-bottom intertidal areas (*i.e*. 0.1 to 0.8 m^2).

It is acknowledged that the original intent and sampling design was to map the fauna of the bay, spatially, and subsequently, temporally, and was not specifically designed for multivariate analysis. Even so, the analyses here demonstrate that even for mapping purposes, the sampling intensity is inadequate and

does not provide robust data on the fauna at each location. The analytical methods employed here (univariate and multivariate techniques) would also commonly be used on AnnaRoeBIM data no matter the design. Plots of changes in species distributions presented by Piersma *et al*. (2016) clearly show spatial variability in taxa distributions, but the noise in these data is not apparent from the plots, and the plots do not portray stations where the species occur but were not captured due to under-sampling.

Elliot (1977) provides comprehensive discussion and guidance on selecting the sampling size unit (surface area) and number of replicates for the estimation of benthic invertebrate populations. As a general rule, the smallest unit size and a large number of replicates will afford the most precise, accurate and representative measures of a contagious population, but there is no definitive rule, and the final choice is typically constrained by the practicalities of field sampling (Elliot 1977, and references therein). One approach for selecting sampling unit size, is to choose a size that minimises the variance in the data (see Elliot 1977, Gonor & Kemp 1978, Andrew & Mapstone 1987). A simplistic method for determining this is to take replicates of different unit sizes, and plot the variance against the relevant unit size. The most appropriate unit size is that which corresponds to peak variance. This approach was used for a cursory investigation of current data. Analyses were performed on MonRoeb16 data from site FP (stations A and B) and site OT (station A), as a larger number of replicate samples were available to test for these single locations; 6 cores per sample per station, with 4 samples for of FP-A and FP-B, and 8 samples for OT-A.

For each station, species data for replicate samples were pooled to provide a series of samples for differing surface areas, *i.e*. 6 cores, 12, 18 and 24 cores, respectively equivalent to 0.05, 0.1, 0.15 and 0.2 m^2. The variance for each was calculated and plotted against the relevant number of cores (Figure 13). For FP-B and OT, 6 cores (0.05 m^2) per site appeared to be an appropriate sample unit size for determining species richness, but 12 (0.1 m^2) per site may be needed to determine abundance. For FP-A, which is closer to shore than site FP-B, and more speciose, the plot showed peaks in variance at 6 cores for both species richness and abundance, with a second peak at 18 cores (0.15 m^2) for abundance. This suggests that a sampling area of 0.05 m^2 per site adequately captured species present in smaller clumps, but there appears to be larger, higher density aggregations of these species that were only captured by sampling a larger area (at least 0.15 m^2). A larger number of cores are needed to determine when maximum variance was reached for abundance at FP-A, and cores adequately captured a representative sample of these larger aggregations.

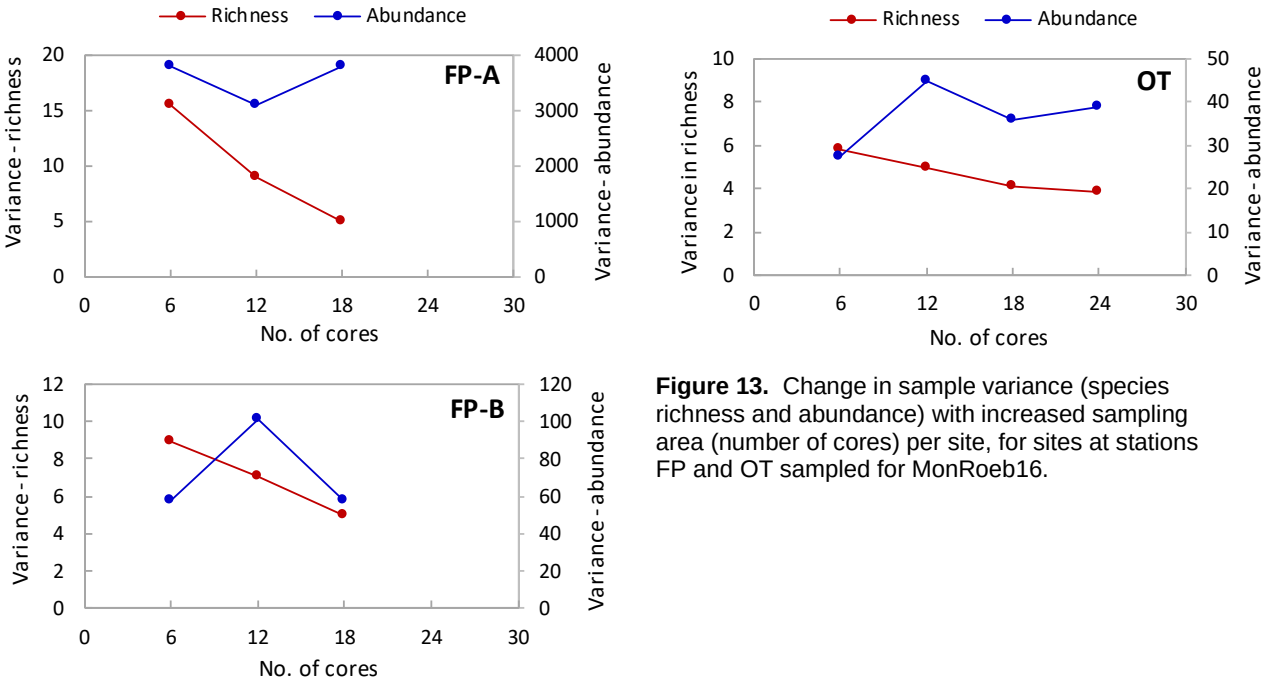


Figure 13. Change in sample variance (species richness and abundance) with increased sampling area (number of cores) per site, for sites at stations FP and OT sampled for MonRoeb16.

To help overcome the potential problem of under-sampling in RB (and EMB), and to reduce the variance in the data for meaningful statistical tests, data from individual AnnRoeBIM-16 samples were pooled. In deciding how many and which samples to pool, consideration was given to the published literature as well as likely confounding effects of inundation and sediment grain size (penetrability) due to pooling spatially separate stations within both RB and EMB. If too many samples were pooled across differing habitat zones, gradients in species assemblages may also be obscured and variance further increased. So, no matter what rule was used for pooling, it necessitated a compromise between providing adequate species coverage and spatially confounding 'samples' by pooling across spatial boundaries.

The method of Anderson and Santana-Garcon (2015) was used to assist in selecting the minimal number of samples to pool for multivariate analysis of community data, based on Bray-Curtis dissimilarity values (refer Methods section 2.2.2.1). Resultant plots showed that pooling ≥ 3 samples (*i.e*. 9 cores) reduced both the number of undefined similarities (due to samples with zero species), and the proportion of samples that were 100% dissimilar, to zero. Examples are provided in Figure 14 for TB and OT. This is supported by histograms of the distribution of dissimilarities which show a considerable reduction in skewness when the number of pooled samples is ≥ 3 (Figure 15).

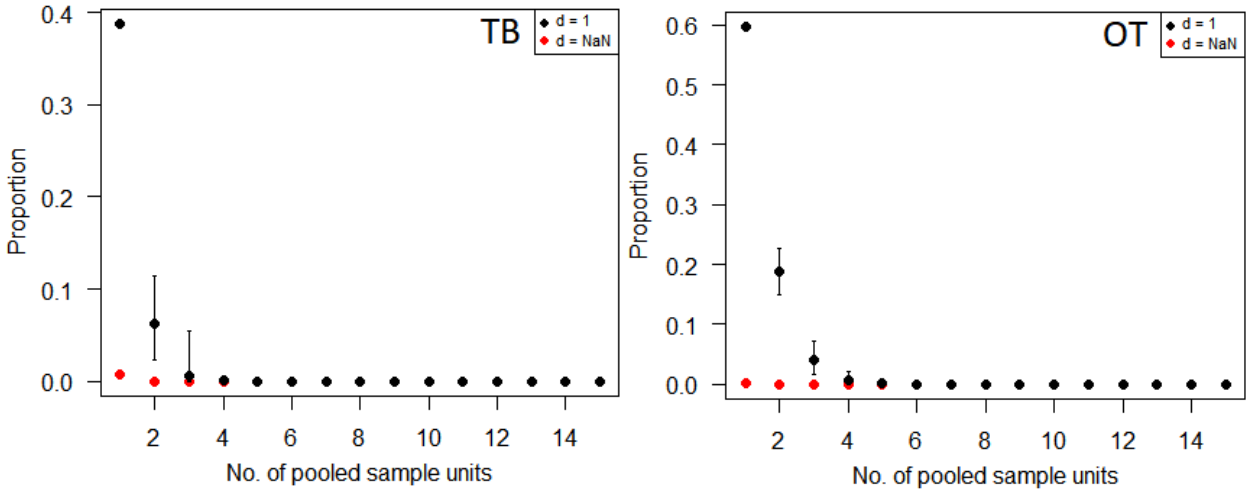


Figure 14. RB 2016: proportion of Bray-Curtis dissimilarities equal to 1.0 (no species in common) or undefined (*i.e*., NaN, "not a number") for TB and OT with increasing numbers of samples being pooled together (from 1 to 14). Error bars indicate the 2.5 and 97.5 percentiles of the distribution of values obtained under 1000 permutations of the order of sampling units.

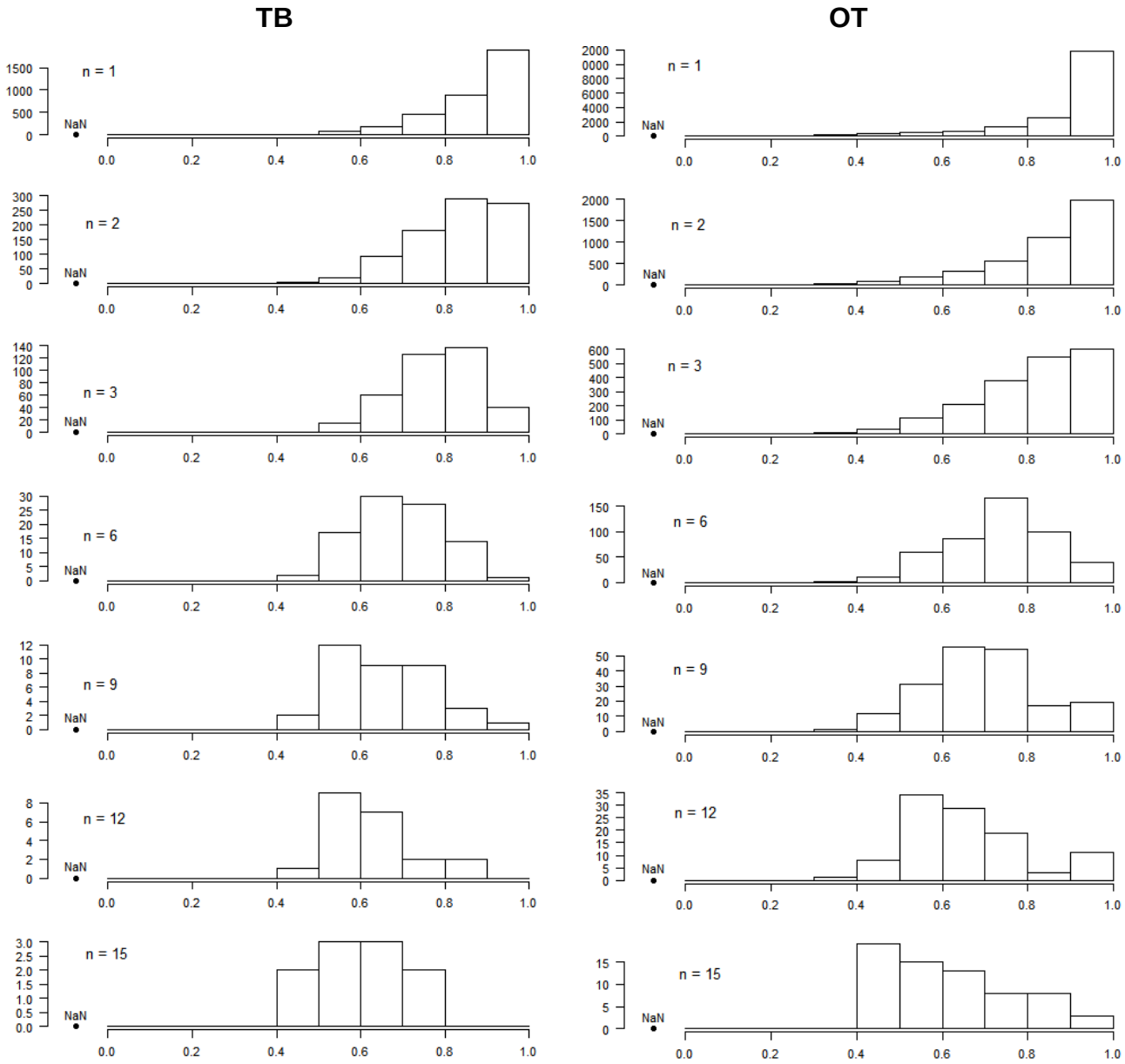
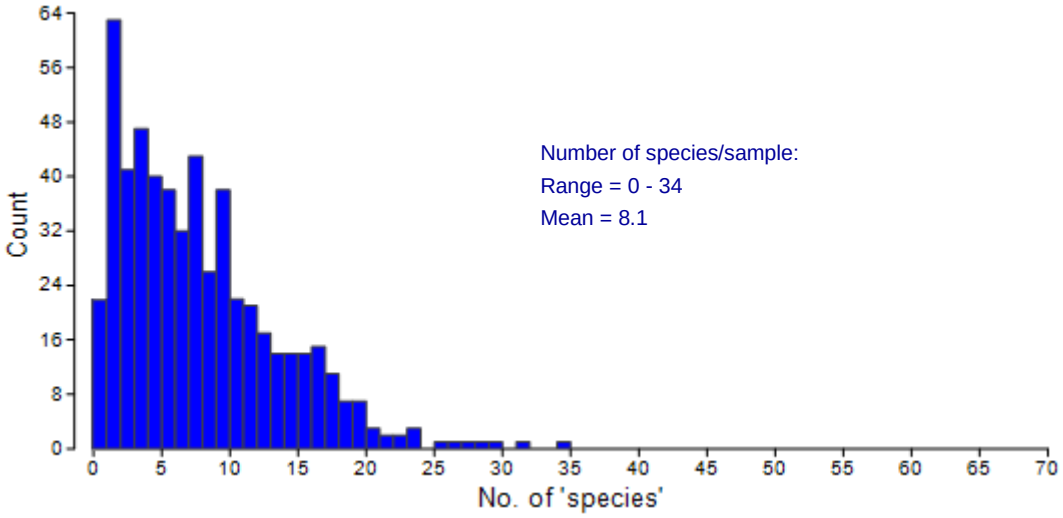


Figure 15. RB 2016: Frequency distributions for Bray-Curtis dissimilarities for increasing numbers of samples from TB and OT being pooled (*n* = 1 to 15). Pooling of samples was done merely in order of sample identification number.

Pooling 3 samples (*i.e*. 9 cores, total surface area = 0.075 m^2) was therefore considered an appropriate compromise between loss of information and highly variable, though typically species-poor, samples. Each pooled sample comprised 3 samples grouped by *a priori* area and sub-area, as described in Methods section 2.2.1. The effects of pooling on species richness are illustrated in Figures 16a-b, as well as in Figure 12B (above). Pooling reduced the number of samples to 128, increased average number of species per sample to 21.2, and reduced the number of samples with few species (*i.e*. < 5 species/sample) to zero (Figure 16a-b). Of the 130,560 pairwise combinations of pooled samples, 320 pairs (*i.e*. 0.24%) had no species in common, compared to 43% of samples in the un-pooled dataset. By way of further example, shade plots of species abundance for RB are provided in Figure 17a-b.

Species accumulation curves for pooled data suggest 9 cores may still under-sample (Figure 18). For all areas, the accumulation curve for the total number of species observed (Sobs) continues to rise, and based on the predictions of several non-parametric (Chao1, 2, Jacknife 1, 2, Bootstrap) and parametric (MM) extrapolators, the probable number of species present in each area was likely to be considerably higher.

(a) Sample size = 3 cores/sample



(b) Pooled samples = 9 cores/sample

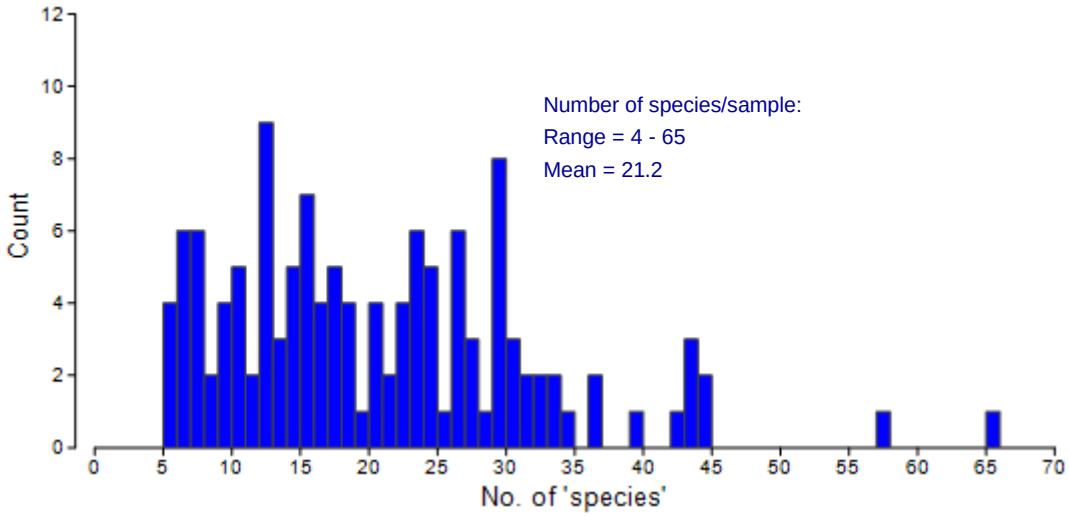


Figure 16. RB 2016: frequency histograms for species richness (number of species) for (a) standard sample size of 3 cores, and (b) pooled sample size of 9 cores. Average and range in values for species richness across samples is also provided.

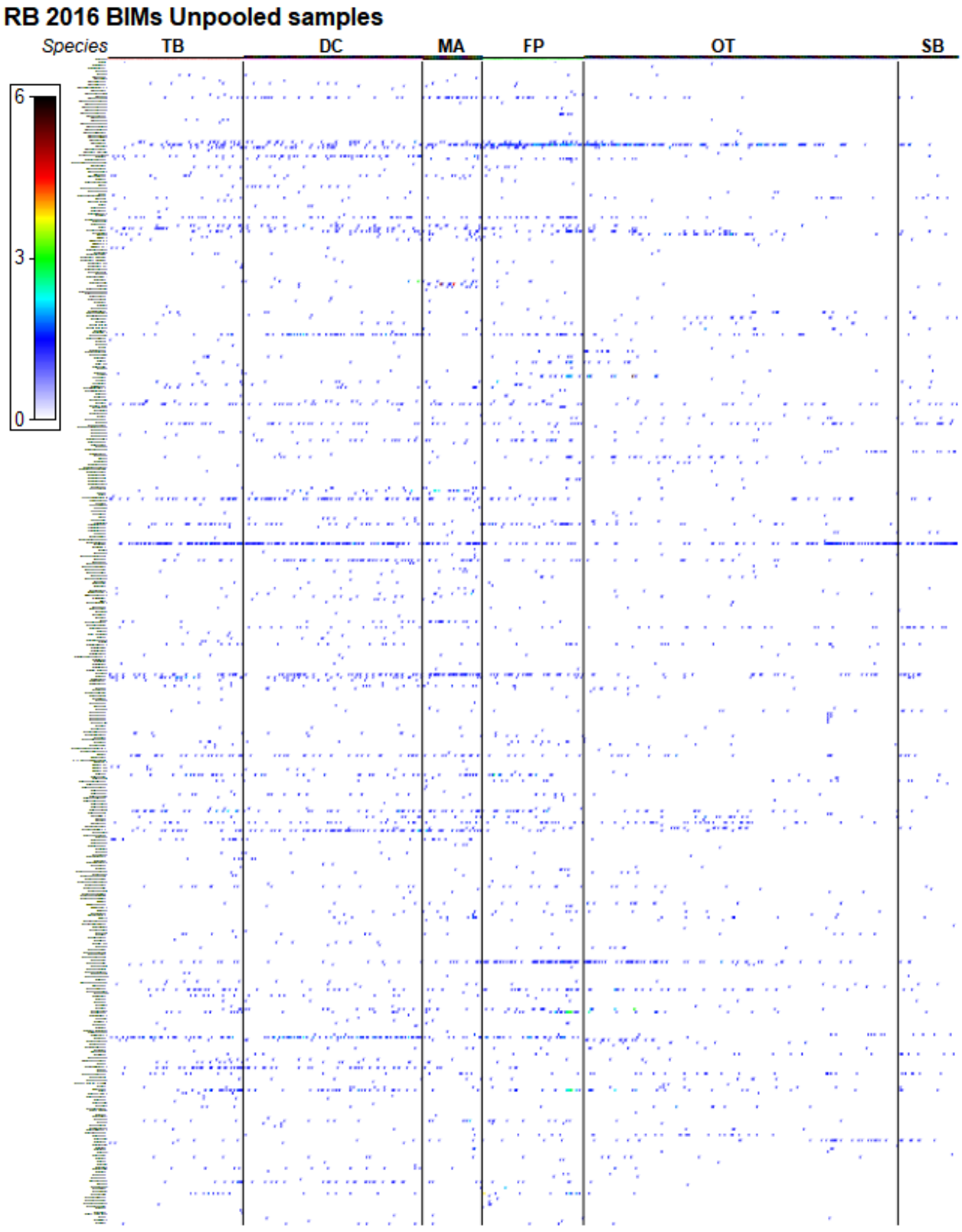


Figure 17a. RB 2016: shade plot for species assemblage data for un-pooled samples, ordered by distance from Town Beach and *a priori* area. Rectangle colours represent abundances (fourth-root transformed) on a continuously linear scale from absent (white) to the maximum value for the matrix (black). Note, species names are not intended to be legible, the gradation in 'shaded' is the relevant attribute to interpret.

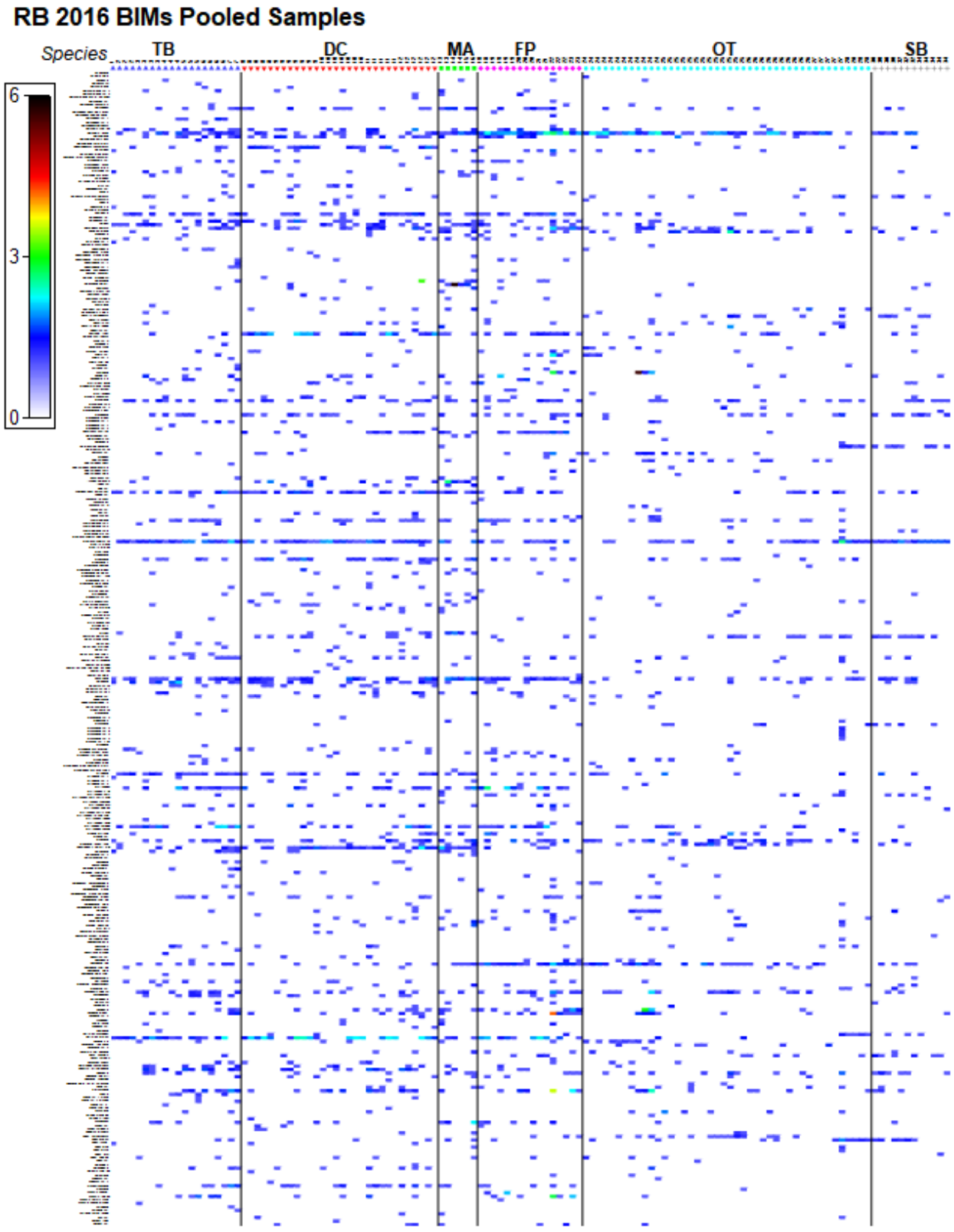


Figure 17b. RB 2016: shade plot for species assemblage data for pooled samples, ordered by distance for Town Beach and *a priori* area. Rectangle colours represent abundances (fourth-root transformed) on a continuously linear scale from absent (white) to the maximum value for the matrix (black). Note, species names are not intended to be legible, the gradation in 'shade' is the relevant attribute to interpret.

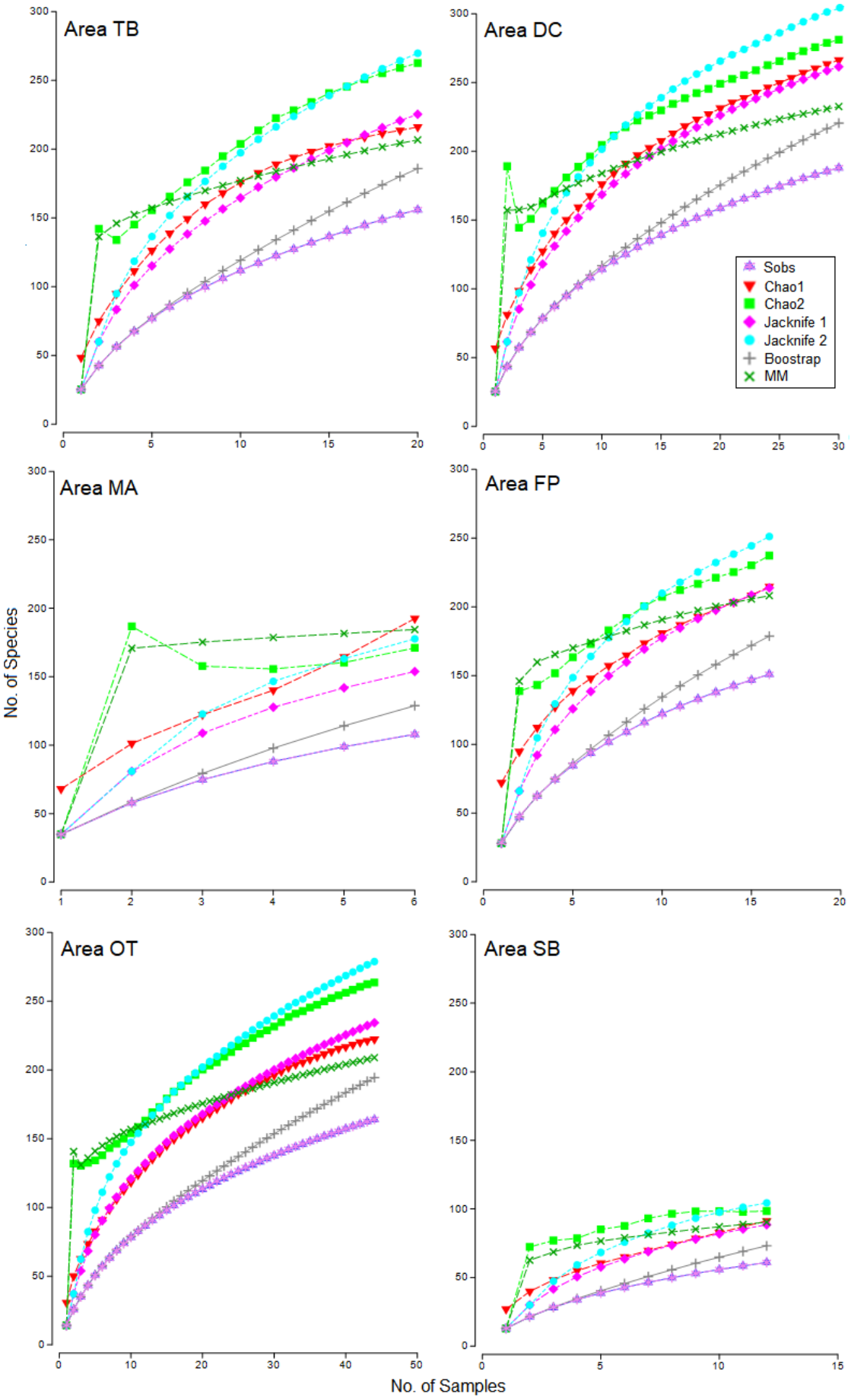


Figure 18. RB 2016: species accumulation curves for each area (after pooling) based observed richness (Sob) and various richness estimators (Chao 1, Chao 2, Jacknife 1, Jacknife 2, Bootstrap, MM) for AnnRoeBIM-16 data.

3.3 Spatial Variability in Biota and Relationships with Environmental Factors

3.3.1 Univariate Metrics

Relationships with environmental variables were investigated using Spearman correlation (Table 1) and linear regression analyses on 2016 data (Table 2), and confirmed with scatter plots of species metrics on environmental data. Scatter plots for pooled as well as raw data are provided in (Appendix 1).

Spearman correlation using combined data for all areas, indicated there were weak negative relationships with distance from Town Beach for species richness, abundance and Shannon Weiner diversity (Table 1), however scatter plots showed these were driven largely by lower values in OT and SB, compared to other areas. For individual areas, stronger negative relationships with distance were found for species richness (rho = -0.77, $p < 0.001$), evenness (rho = -0.88, $p < 0.001$) and diversity (rho = -0.90, $p < 0.001$) at SB, but in contrast, at TB, positive relationships with distance were found for species richness (rho = -0.87, $p < 0.001$), abundance (rho = -0.75, $p < 0.001$) and diversity (rho = -0.75, $p < 0.001$) (see Appendix 1).

Moderate positive relationships were also found for total seagrass cover and species richness (rho = 0.52, $p < 0.001$) and diversity (rho = 0.57, $p < 0.001$), using combined data for all areas (Table 1). Cover of both linear seagrass (*Halodula uninervis*) and oval seagrass (*Halophila ovalis*) were significantly correlated with these metrics, though the relationship was slightly stronger (rho = 0.50) for linear seagrass (Table 1). There also appeared to be a moderate positive relationship between species abundance and algal cover (rho = 0.554, $p < 0.001$) (Table 1). A number of other statistically significant relationships were detected, though these were relatively weak, as indicated by the low rho values (rho ≤ 0.33). For example, there were weak, negative relationships for species abundance and inundation, qualitative inundation category (0-30, 31-70, 71-100%) and qualitative penetrability category (0-10, 11-20, 21-30, 31-40cm) (Table 1).

Table 1. RB 2016: significant results from Spearman correlation analyses for species diversity measures and environmental parameters, after pooling. Significant *p* values ($p < 0.05$) are highlighted blue for clarity.

Environmental parameter	No. of samples	Correlation	Species richness (*S*)	Total abundance (*N*)	Evenness (*J'*)	Diversity (*H'* $\log_e$)
Distance (m)	128	Spearman rho	-0.51	-0.45	-0.07	-0.50
		p value	<0.001	<0.001	0.452	<0.001
Inundation	128	Spearman rho	-0.17	-0.26	0.14	-0.07
		p value	0.061	0.003	0.102	0.407
Inundation_category	128	Spearman rho	-0.16	-0.23	0.12	-0.08
		p value	0.079	0.009	0.169	0.343
Penetrability_category	93	Spearman rho	-0.17	-0.24	0.12	-0.07
		p value	0.096	0.019	0.272	0.526
Seagrass_H.oval.	128	Spearman rho	0.41	0.24	0.18	0.48
		p value	<0.001	0.007	0.048	<0.001
Seagrass_H.unin.	128	Spearman rho	0.50	0.29	0.21	0.56
		p value	<0.001	0.001	0.016	<0.001
Total_seagrass	128	Spearman rho	0.52	0.33	0.18	0.57
		p value	<0.001	<0.001	0.039	<0.001
Algae	128	Spearman rho	0.50	0.55	-0.19	0.31
		p value	<0.001	<0.001	0.033	<0.001
Algae + Seagrass	128	Spearman rho	0.63	0.52	0.06	0.58
		p value	<0.001	<0.001	0.492	<0.001

Again, stronger relationships with penetrability and inundation were found within individual areas (see Appendix 1). At TB, penetrability was strongly correlated with species richness (rho = 0.71, *p* = 0.001), diversity (rho = 0.72, *p* = 0.001), and to a lesser degree abundance (rho = 0.56, *p* = 0.01), while inundation was negatively correlated with abundance (rho = -0.58, *p* = 0.007). At OT, penetrability was also negatively correlated with abundance (rho = -0.78, *p* = 0.005). At SB, relatively strong positive correlations with inundation were found for species evenness (rho = 0.80, p = 0.002), diversity (rho = 0.65, *p* = 0.022), abundance (rho = 0.62, *p* = 0.032) and richness (rho = 0.59, *p* = 0.042).

Stepwise multiple regression indicated algae and distance to have the best linear relationship with species richness, together accounting for 23.5% of total variance (Table 2). Algae alone showed the best linear relationship with total abundance, though it only accounted for 8.1% of total variance. The combination of parameters that explained the most (26.1%) of the total variance in species evenness were algae, linear seagrass, penetrability category and distance (Table 2). For species diversity, the combination of parameters that explained the most of the total variance (29.7%) were linear seagrass, distance, penetrability category and inundation. There were no other significant linear relationships between species metrics and the measured environmental parameters (as expected based on the results of the Spearman correlations). The unique variance explained by continuous data for penetrability and qualitative inundation category (squared partial correlations; not shown) was low for all species metrics: < 6% and < 7%, respectively.

Table 2. RB 2016: significant results from stepwise multiple regression of species diversity measures on environmental parameters, after pooling. R = correlation value; %Var. = percentage of variance in species data explained.

Dependent	Independents	Model	ANOVA				R	%Var.
			df	MS	F	*p*		
Species Richness (*S*)	Algae, Distance	Regression	2	1167	13.792	<0.001	0.484	23.5
		Residual	90	85	--	--	--	--
		Total	92	--	--	--	--	--
Total Abundance (*N*)	Algae	Regression	1	2400093	8.019	0.006	0.285	8.1
		Residual	91	29938	--	--	--	--
		Total	92	--	--	--	--	--
Evenness (J')	Algae, Seagrass_linear, Penetrability_cat, Inundation	Regression	4	0.108	7.755	<0.001	0.511	26.1
		Residual	88	0.014	--	--	--	--
		Total	92	--	--	--	--	--
Diversity (*H'* $\log_e$)	Seagrass_linear, Distance, Penetrability_cat, Inundation	Regression	4	2.189	9.316	<0.001	0.545	29.7
		Residual	88	0.224	--	--	--	--
		Total	92	--	--	--	--	--

ANCOVA including inundation and penetrability as covariables was used to test for significant differences in species metrics among *a priori* areas. Results indicated there were significant differences among areas for most of the species metrics (except richness), when inundation and penetrability were controlled for. Significant inundation effects were also apparent, but there were no significant penetrability effects (Table 3). Average species richness and abundance were significantly lower in areas SB (12.9 $\pm$ 1.60 SE and 28.0 $\pm$ 3.88 SE, respectively) and OT (14.2 $\pm$ 1.29 SE and 61.1 $\pm$ 24.0 SE, respectively) than in most other areas, but both indices were significantly higher in MA (34.8 $\pm$ 6.62 SE and 286.5 $\pm$ 183.0 SE, respectively) than in most other areas. Average diversity was also significantly lower at OT (2.0 $\pm$ 0.08 SE) than most other areas (except SB, 2.2 $\pm$ 0.14 SE), but there were no statistically significant differences in diversity among other areas. Within-area variability in species richness, abundance and diversity was particularly high for MA and evenness correspondingly low (ave. 0.74 $\pm$ 0.13 SE). Plots in Figure 19a illustrate the differences in average values for species metrics among areas, together with 95% confidence

intervals. Inundation effects were not consistent across areas (evidenced by the significant interaction term, Area x Inund, $p < 0.001$), but pairwise comparisons (not shown) suggested that in general, species richness, abundance and diversity, and to a lesser degree evenness, tended to be significantly lower in zones inundated for long periods (*i.e.* > 70 % of the time) compared to medium (31 - 70%) or shorter (≤ 30%) periods.

ANCOVA testing also showed there were no significant sub-area, inundation or penetrability effects within each area. However, samples sizes for most sub-areas (except DC) were too small to be confident of results. Plots of average values for species metrics for each sub-area are provided in Figure 19b.

Table 3. ANCOVA testing for significant ($p < 0.05$) Area effects on RB 2016 species richness, abundance (log_{10} transformed), diversity and evenness, after pooling, including inundation (Inund) and penetrability (Penetr) as covariables. Significant *p* values are highlighted blue for clarity.

Source	ANCOVA (main effects)				ANCOVA (main effects)			
	df	**MS**	**F**	***p***	**df**	**MS**	**F**	***p***
	Species richness				**Species abundance**			
Inundation	1	11.3	0.235	0.630	1	0.94	19.094	<0.001
Penetrability	1	61.1	1.271	0.264	1	0.01	0.172	0.680
Area	5	112.4	2.339	0.051	5	0.23	4.394	0.002
Inund x Penetr	1	234.6	4.883	0.030	1	985300	0.002	0.964
Area x Inund	5	98.8	2.056	0.081	5	0.30	6.103	<0.001
Area x Penetr	5	125.0	2.601	0.033	5	0.06	1.309	0.270
Area x Inund x Penetr	5	104.7	2.180	0.066	5	0.07	1.353	0.253
Residual	69	48.1	--	--	69	0.05	--	--
Total	93	--	--	--	93	--	--	--
	Diversity (*H'* log_e)				**Evenness (*J'*)**			
Inundation	1	1.11	9.376	0.003	1	0.018	30.715	<0.001
Penetrability	1	0.19	1.572	0.214	1	0.001	1.522	0.222
Area	5	0.48	4.050	0.003	5	0.003	5.511	<0.001
Inund x Penetr	1	0.54	4.584	0.036	1	0.001	2.341	0.131
Area x Inund	5	1.16	9.791	<0.001	5	0.008	13.763	<0.001
Area x Penetr	5	0.23	1.916	0.103	5	0.000	0.670	0.647
Area x Inund x Penetr	5	0.21	1.743	0.136	5	0.001	0.872	0.504
Residual	69	0.12	--	--	69	0.001	--	--
Total	93	--	--	--	93	--	--	--

Seagrass effects were examined separately for those areas where seagrass occurred, and within which there were a sufficient number of replicate samples to test between zones with and without seagrass, *i.e.* TB, DC and FP. Two-factor ANOVA testing (Area x Total Seagrass) showed no significant seagrass effect for any of the species metric ($p \geq 0.13$), and the non-significant interaction terms ($p \geq 0.083$) indicated this was consistent across areas.

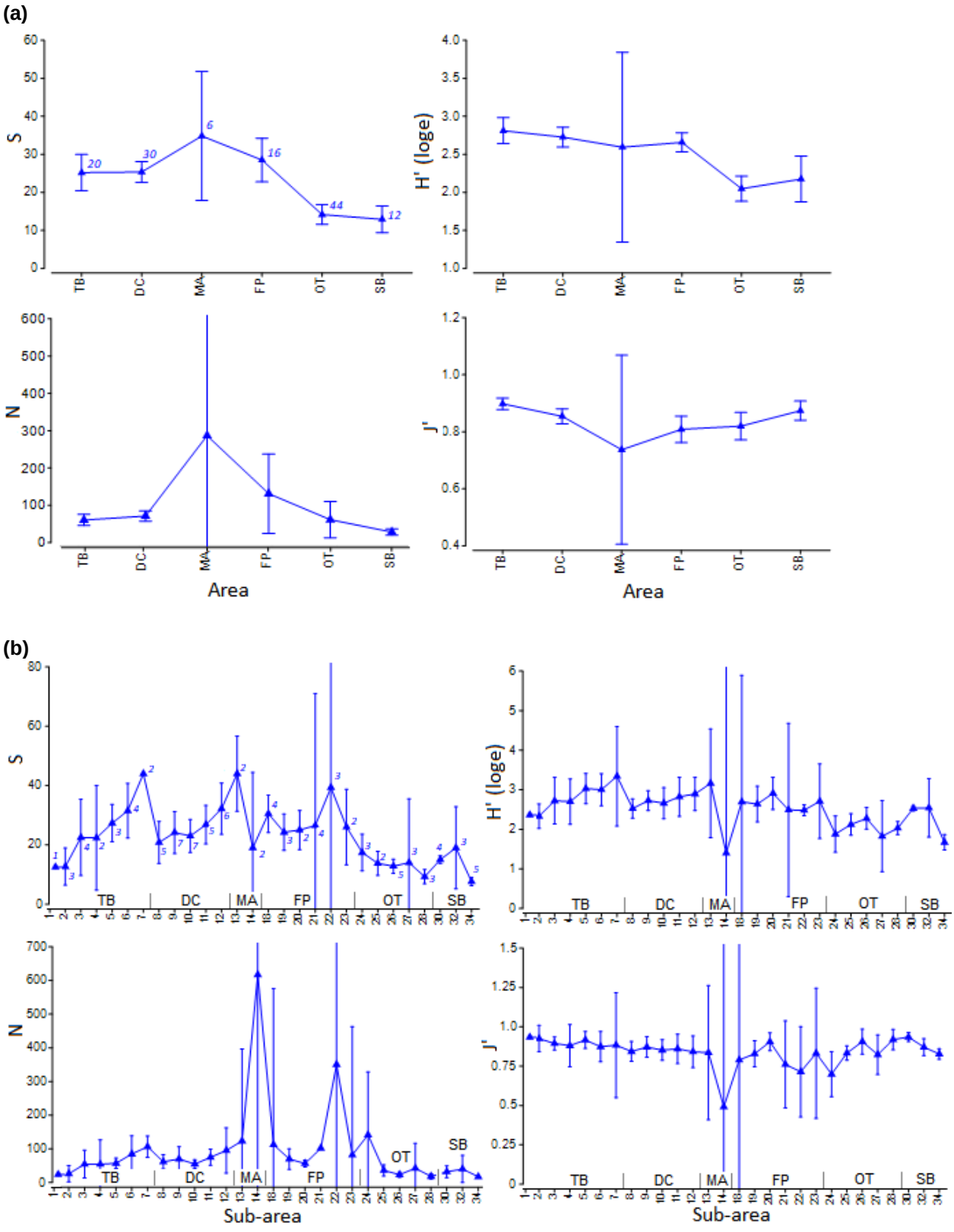


Figure 19. RB 16: Diversity indices (average ± 95% CI) for species-level data (after pooling) for, (a) each *a priori* area, and (b) each *a priori* sub-area. *S* = total number of species; *N* = total number of individuals; *H'* $\log_e$ = Shannon-Wiener diversity; *J'* = Pielou's evenness index. Number of samples after pooling is provided on each of the species richness plots.

3.3.2 Multivariate Metrics

Exploratory analysis using k-R clustering and the SIMPROF test on both pooled and un-pooled samples showed large variability in species assemblages, but that the benthic invertebrate community could be broadly divided into four groups ($p < 0.05$) which loosely corresponded to geographic location and inundation zone (Figure 20). While there were obvious differences between un-pooled and pooled samples, the major groupings were similar: i) short to median inundation zones from Town Beach to Fall Point, ii) longer inundation zones of Town Beach and Dampier Creek, iii) most of mid to outer flats of the One Tree area, and iv) inner One Tree and Southern Beach.

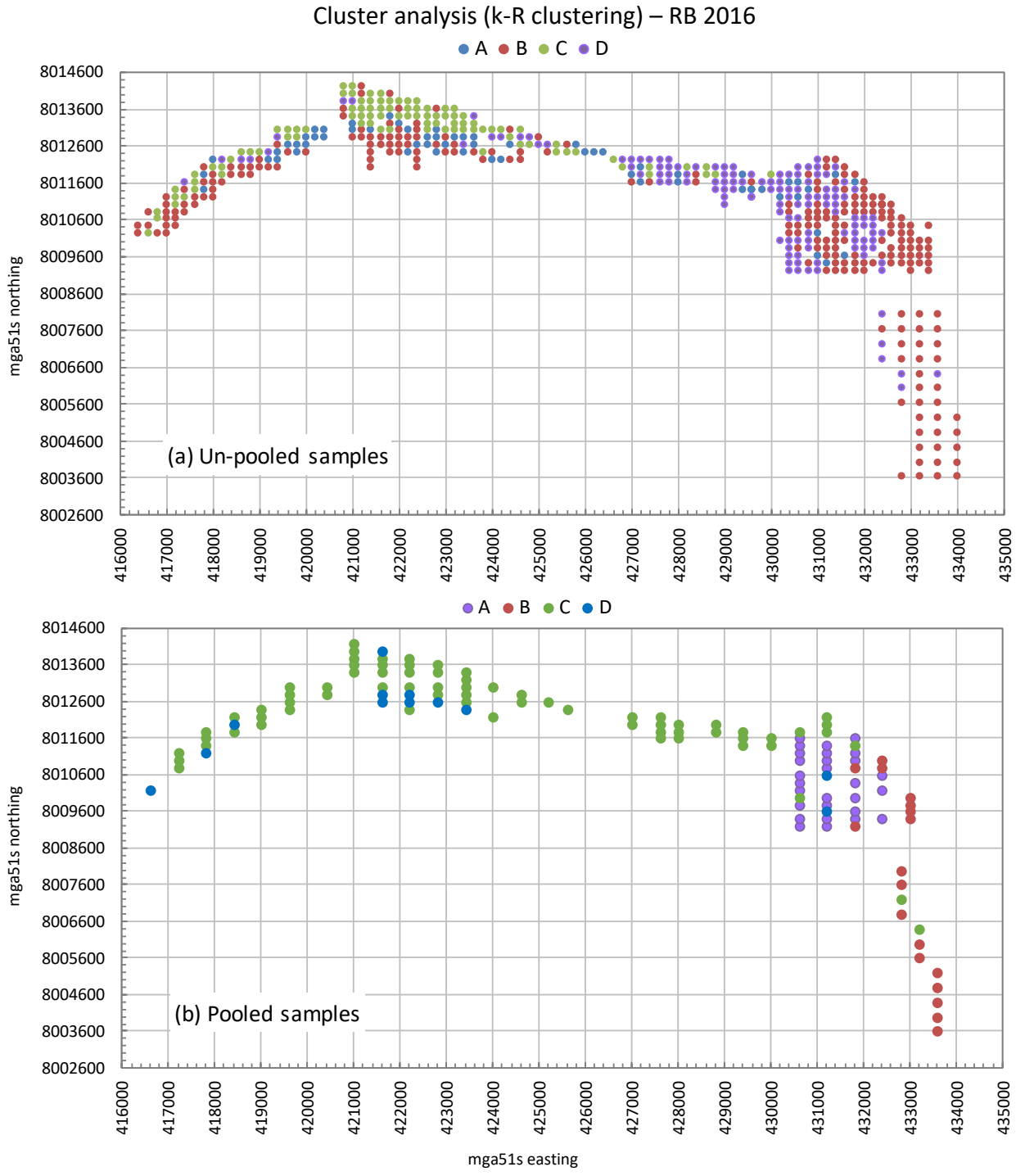


Figure 20. RB 2016: station groupings (A, B, C, D) determined from k-R clustering (non-hierarchical) on species assemblages (4th root transformed abundance), based on Bray-Curtis similarity for (a) un-pooled samples, and (b) pooled samples.

Multivariate multiple regression analysis (DistLM) identified a number of environmental parameters with significant (p = 0.001) relationships with species assemblages (Table 4). The parameter that individually explained the greatest amount of variation in the species data was distance from Town Beach (7.13%), penetrability (6.71%), followed by inundation (6.29%), linear seagrass cover (5.94%), oval seagrass cover (4.3%) and algal cover (3.1%); together accounting for a maximum 22.11% of the total variation. Qualitative categories for inundation (0-30, 31-70, 71-100%) and penetrability (0, 10, 20, 30, 40cm) explained slightly less of the total variation than did continuous data for these parameters, *i.e*. 6.0% and 5.71% respectively (Table 4).

Table 4. Results from multivariate multiple regression (DistLM) of RB 2016 species assemblage data on environmental parameters for (a) each parameter individually, and (b) stepwise selection of parameters. %Var. = percentage of variance in species data explained; Cum.% = cumulative percentage of variance explained.

Parameter	Pseudo F	*p*	%Var.	Cum.%
(a) Parameters individually				
Distance(m)	6.991	0.0001	7.13	
Penetrability	6.541	0.0001	6.71	
Inundation	6.112	0.0001	6.29	
Penetrability_category	5.813	0.0001	6.00	
Seagrass_H.unin.	5.748	0.0001	5.94	
Inundation_category	5.516	0.0001	5.71	
Total_Seagrass	5.410	0.0001	5.62	
Seagrass+Algae	5.289	0.0001	5.49	
Seagrass_H.oval.	4.093	0.0001	4.30	
Algae	2.909	0.0001	3.10	
(b) Parameters fitted sequentially				
Distance(m)	6.991	0.0001	7.13	7.13
Inundation	7.450	0.0001	7.10	14.23
Algae	3.741	0.0001	3.46	17.69
Seagrass_linear	2.626	0.0001	2.38	20.08
Penetrability	2.274	0.0002	2.04	22.11

Despite each only explaining a very low percentage of the total variation, penetrability, inundation and seagrass cover appeared to have a significant influence on species assemblages within areas. In Figure 21a, the dbRDA ordination plot for species abundance data versus environmental data shows samples separated by penetrability and inundation along the x-axis (dbRDA1 axis), and by seagrass cover along the y-axis (dbRDA2 axis). Samples from relatively higher penetrability and inundation zones tend toward the right side of the plot, and samples with greater seagrass cover toward the top of the plot (Figure 21a). Species best correlated with the ordination axes were the tellinid bivalve *Serratina piratica* (Pearson r = 0.7), which appeared to be more common in lower penetrability zones, and the solemyid bivalve *Solemya terraereginae* (Pearson r = 0.67) which was more common in zones with greater seagrass cover (Figure 22b). Weaker relationships (0.5 < r < 0.6) were observed for several other taxa such as the lucinid bivalve *Divaricella irpex* and Nephytidae polychaetes in lower penetrability zones, 'Ingrid-eating' snails *Nassarius dorsatus* in higher penetrability-low inundation zones, Sternaspidae polychaetes in higher penetrability-higher inundation zones, and the gastropod *Heterocardia gibbosula* which was only recorded from areas OT and SB (Figure 22b). The distribution of these species around the bay is shown in Figures 23 and 24. Caution must be used when interpreting the dbRDA plot, as the percentage of variation explained by each of the axes was very low (< 10% of total variation). The plot is included primarily to help visualise the relative importance of the environmental parameters to the patterns of variation in the species assemblage data.

The BIO-ENV model explained a similar percentage (23.4%) of the variance in species composition data as the DistLM model, and selected inundation, penetrability and distance from Town Beach as the best combination of 'predictors' (Spearman rho = 0.484, p = 0.001). Of the individual variables, distance was best correlated with the species data, accounting for 14.2% of the variance in the species data.

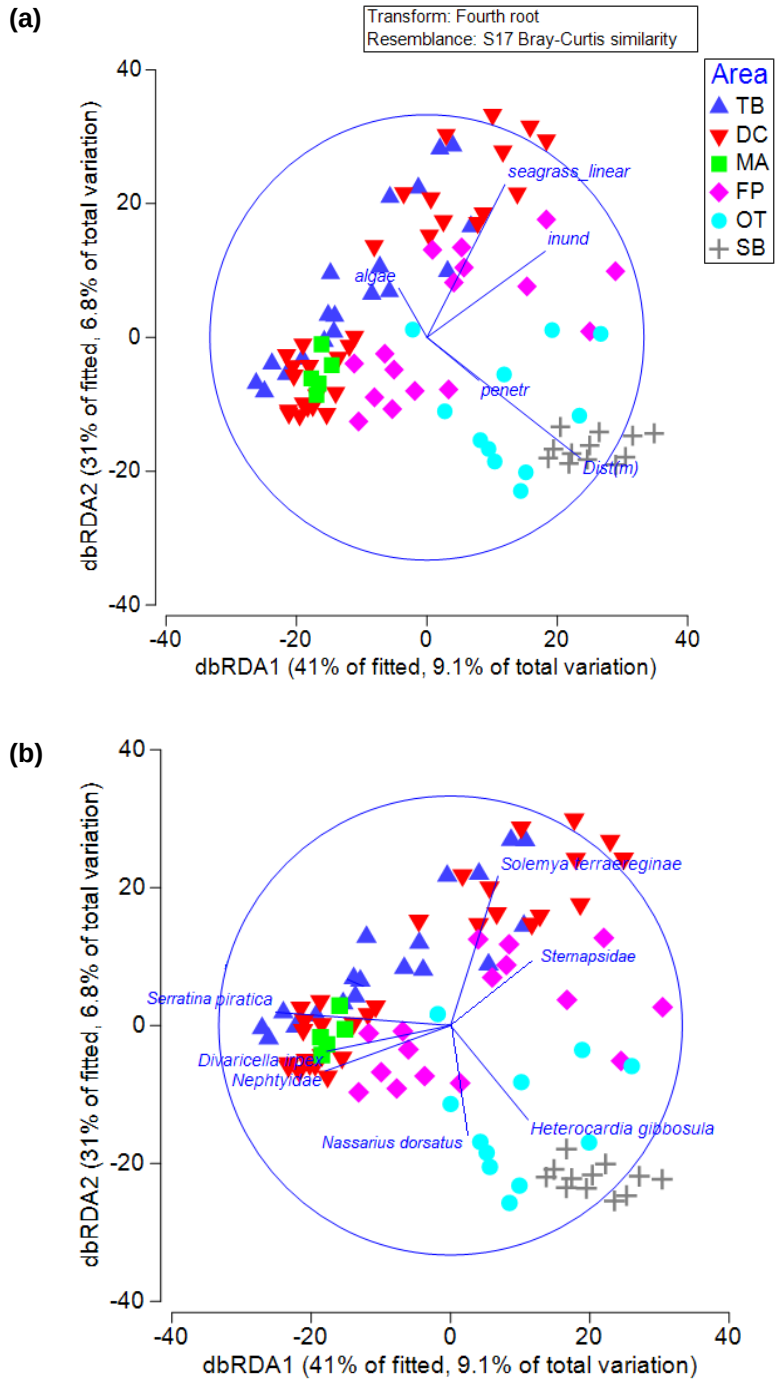


Figure 21. RB 2016: dbRDA ordination for the fitted model on species abundance data versus environmental variables : (a) with vector overlays for penetrability (penetr), inundation (inund), seagrass cover (*H. uninervis*), algal cover (algae) and distance (Dist(m)); (b) with vector overlays of individual taxa best correlated with the ordination axes (Pearson coefficient: 0.50 < r < 0.72).

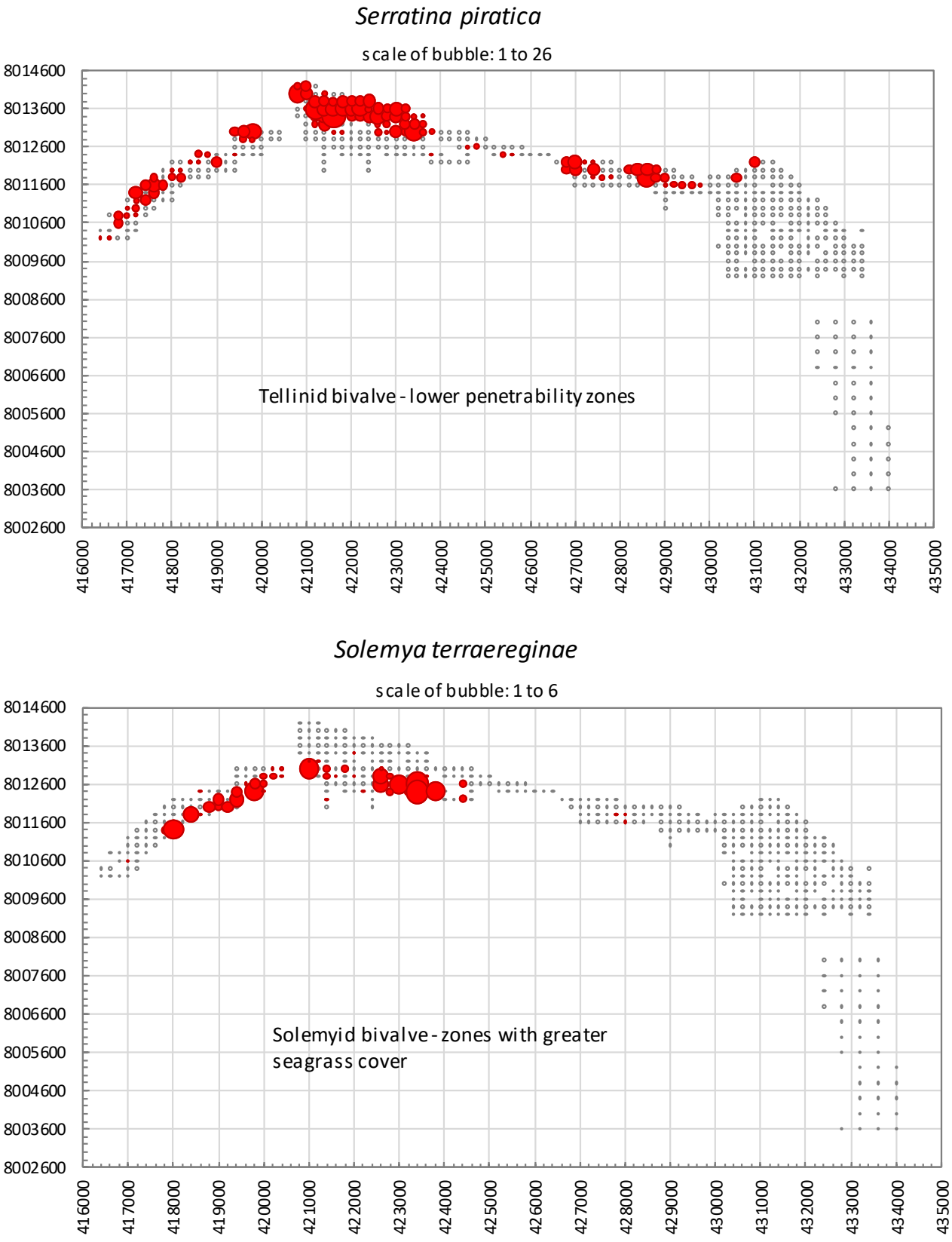


Figure 22. RB 2016: distribution and abundance of the tellinid bivalve *Serratina piratica* and the solemyid bivalve *Solemya terraereginae*.

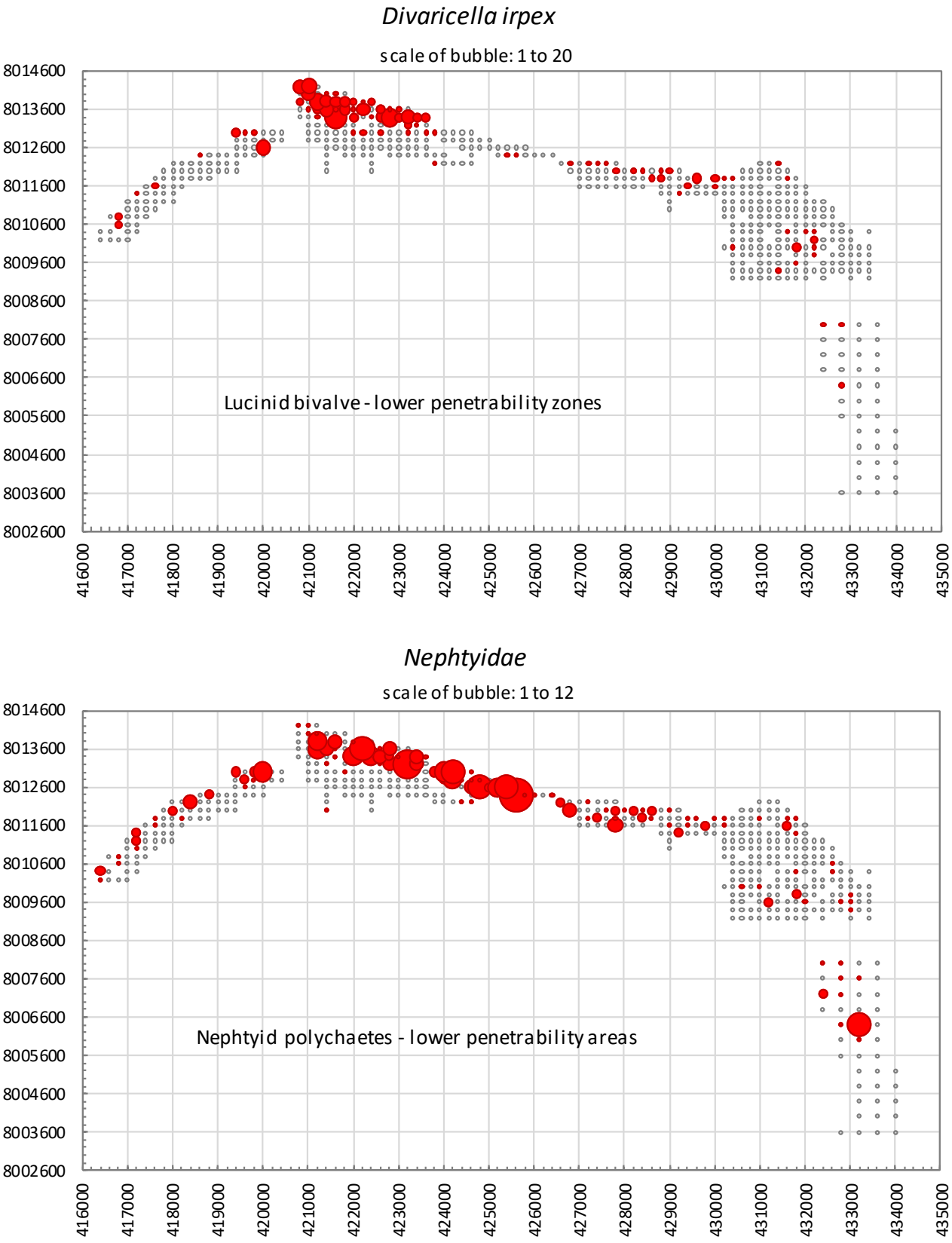


Figure 23. RB 2016: distribution and abundance of the lucinid bivalve *Divaricella irpex* and Nephtyidae polychaetes.

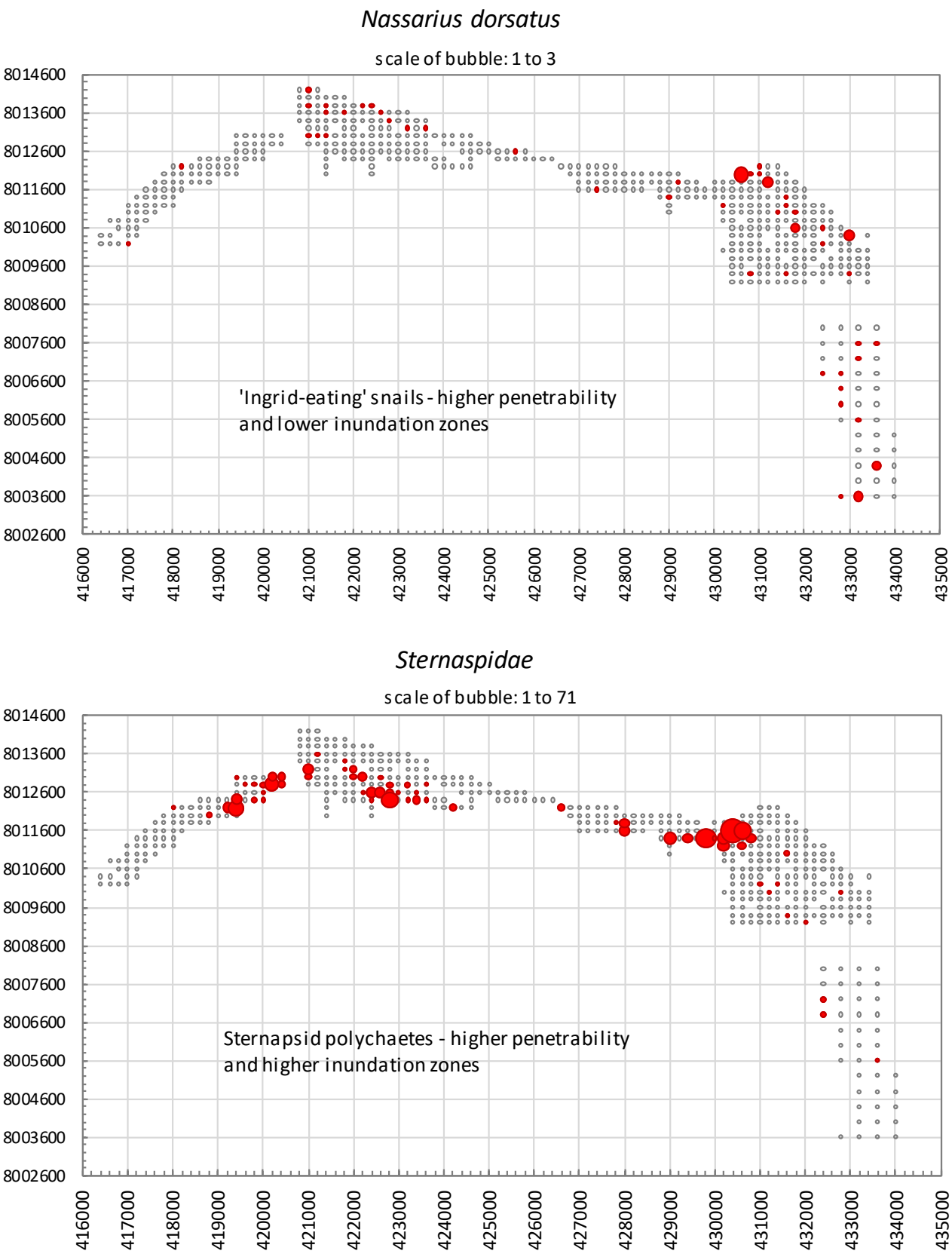


Figure 24. RB 2016: distribution and abundance of the lucinid bivalve *Divaricella irpex* and Sternapsidae polychaetes.

PERMANOVA indicated there were significant area effects on species assemblages, when inundation and penetrability were included as covariables (Table 5). Pairwise comparisons (not shown) indicated all *a priori* areas were significantly different from each other ($p \leq 0.004$). Both penetrability and inundation were statistically significant and the strength of the effect was only slightly greater for inundation (indicated by the relative size of the estimated components of variation, *i.e.* inundation 141.7, penetrability 116.9, Table 5). However, neither inundation nor penetrability effects were consistent across areas, as evidenced by the significant interaction terms (Area x Inund, $p = 0.001$; Area x Penetr, $p = 0.001$). Effects of penetrability were also variable for inundation (Inund x Penetr, $p = 0.001$) but the three-way interaction was not significant (Area x Inund x Penetr, $p = 0.161$).

Inundation and penetrability effects were less important components of the total variation among samples than were area effects (520.7), and all three were small components in comparison to the large amount of unexplained variation as indicated by the relatively large Residual (2147.6) (Table 5).

Table 5. PERMANOVA testing for significant ($p < 0.05$) area effects on RB species assemblages (4th root transformed abundance) including inundation (Inund) and penetrability (Penetr) as covariables.

Source	PERMANOVA main effects				Estimated Components of Variation	
	df	MS	Pseudo-F	*p*	Var.	SD
Inundation	1	15818	5.9834	0.001	141.7	11.9
Penetrability	1	17018	2.749	0.001	116.9	10.8
Area	5	8901	4.1449	0.001	520.7	22.8
Inund x Penetr	1	4691	2.1843	0.002	43.1	6.6
Area x Inund	4	4119	1.9181	0.001	146.3	12.1
Area x Penetr	5	3196	1.488	0.001	252.3	15.9
Area x Inund x Penetr	4	2455	1.1432	0.161	78.0	8.8
Residual	71	2148	--	--	2147.6	46.3
Total	92	--	--	--	--	--

Similarity in fauna both between and within areas was relatively low. Average pairwise percent similarity (Bray-Curtis) between areas ranged from 14.4%, between MA and SB, to 26.2%, between TB and DC (Table 6). Between-area differences were due to relatively small (< 4.5%) changes in abundance of a relatively large (~30) number of species. Average pairwise percent similarity within areas ranged from 26.8% for OT to 36.4% for MA (Table 6).

Table 6. RB 2016: Average (± SE) pairwise percent Bray-Curtis similarity between/within areas.

	TB	DC	MA	FP	OT	SB
TB	28.8 (0.67)					
DC	26.2 (0.40)	28.5 (0.65)				
MA	24.9 (0.75)	24.8 (0.69)	36.4 (3.20)			
FP	26.1 (0.46)	24.8 (0.39)	26.7 (0.69)	32.2 (0.80)		
OT	19.0 (0.27)	17.4 (0.22)	19.7 (0.43)	25.0 (0.32)	26.8 (0.34)	
SB	16.6 (0.51)	15.6 (0.40)	14.4 (1.06)	16.7 (0.64)	17.6 (0.50)	31.5 (1.31)

PERMANOVA testing also indicated there were significant differences within area OT, but not within other areas (Appendix 3). Pairwise tests and interaction terms suggested the differences within OT were related to inundation effects. Samples from sub-area 24, much of which is inundated > 70% of the time (refer section 3.1 Figure 8), appeared to support a significantly different fauna assemblage compared to sub-areas that were mostly inundated for shorter periods, *i.e*. 0 - 30% of the time (sub-area 25 and 26) and 31 - 70% of the time (sub-area 27 and 28). There were no significant differences among sub-areas 25 to 28.

The plot of the mMDS ordination of distances among centroids for areas, together with plots of mMDS ordinations of distances among centroids for penetrability and inundation categories for areas, is shown in Figure 25. The plots for penetrability and inundation visually represent the interaction terms from the PERMANOVA analyses, *i.e.* Area x Penetrability and Area x Inundation, and contrast with the 'Area' plot, which is essentially a plot of the 'main effects' for Area.

The plot of mMDS ordination of distances among centroids for sub-areas (Figure 26a) illustrates the patterns indicated by the PERMANOVA results and shows the large degree of overlap between sub-areas from areas TB and DC. Samples from these areas also group closer to area FP, than to MA, OT or SB. A longitudinal gradient in species assemblages is also apparent around the bay, from sub-area 1 in area TB to sub-area 34 in SB. This gradient is disrupted in area MA, likely due to the lower number of samples (after pooling) from this area (Figure 26b).

Seagrass effects were examined for those areas where seagrass occurred, and within which there were a sufficient number of replicate samples to test between zones with and without seagrass, *i.e*. TB, DC and FP. While there was a significant seagrass effect, this was not consistent across all three areas (PERMANOVA, Table 7). Pairwise tests (not shown) indicated that within areas TB and DC, the presence of seagrass, regardless of density, was associated with a slight but statistically significant difference in species composition compared to zones devoid of seagrass. There were no detectable seagrass effects within area FP.

Table 7. Two-factor PERMANOVA testing for significant ($p < 0.05$) area and seagrass effects on RB species assemblages (4^{th} root transformed abundance) in 2016.

Source	PERMANOVA main effects					Estimated Components of Variation	
	df	SS	MS	Pseudo F	*p*	Var.	SD
Total_seagrass	1	13636	13636	5.871	0.001	171.4	13.1
Area	2	16380	8190	3.546	0.001	277.5	16.7
Area x Total_seagrass	2	8928	4464	1.932	0.002	117.7	10.8
Residual	60	139000	2310	--	--	2309.9	48.1
Total	65	178000	--	--	--	--	--

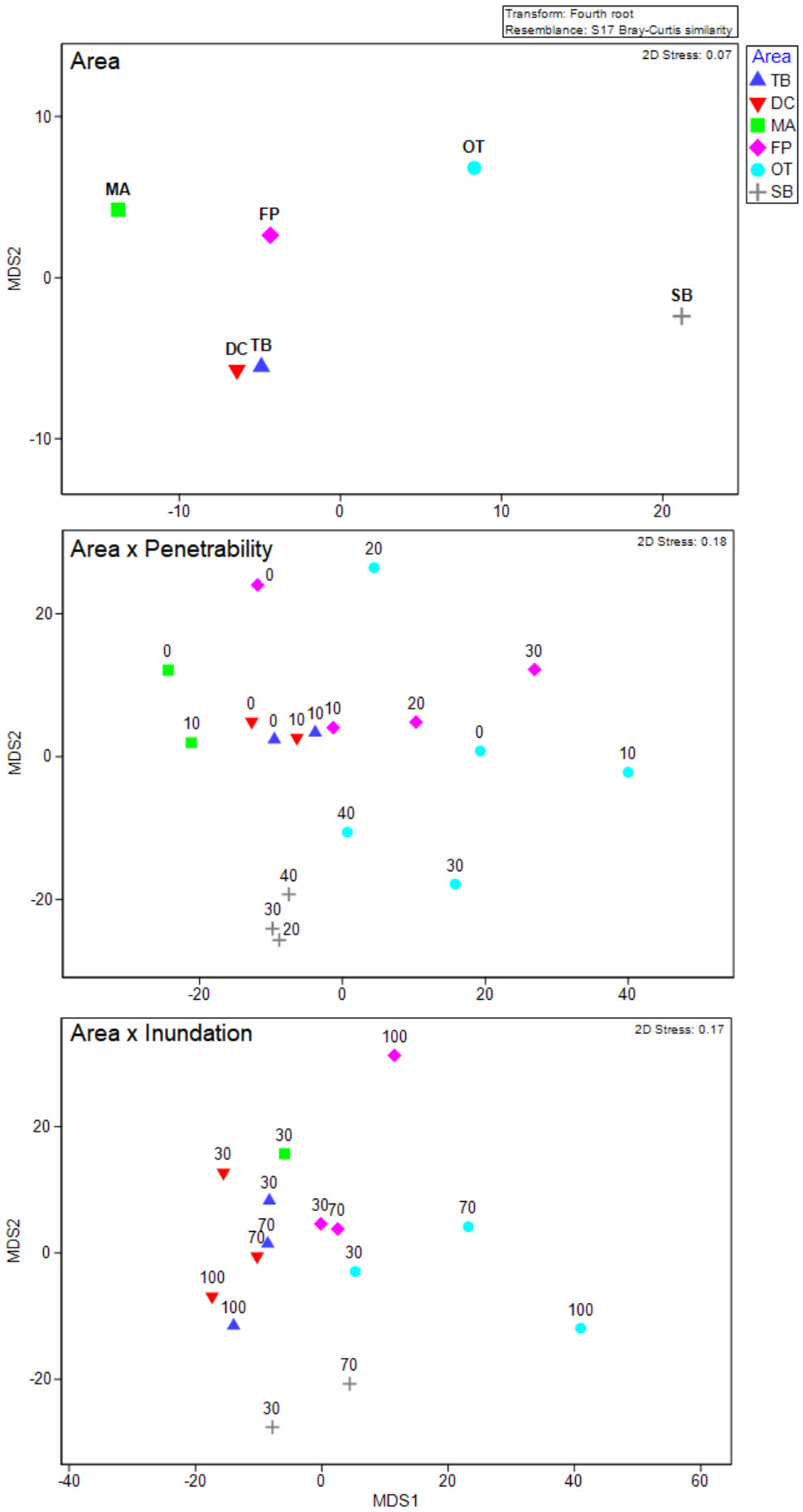


Figure 25. RB 2016: mMDS ordination of distances among centroids for areas (top), penetrability categories (0, 10, 20, 20, 40 cm) for each area (middle) and inundation categories (30 = 0-30%, 70 = 31-70%, 100 = 71-100%) for each area (bottom), based on Bray-Curtis similarity (4th root transformed abundance). Samples are colour-coded by area and labelled by penetrability or inundation category. Optimum solution in 3D for Area with stress = 0.01, Area x Penetrability with stress = 0.11, and Area x Inundation with stress = 0.08.

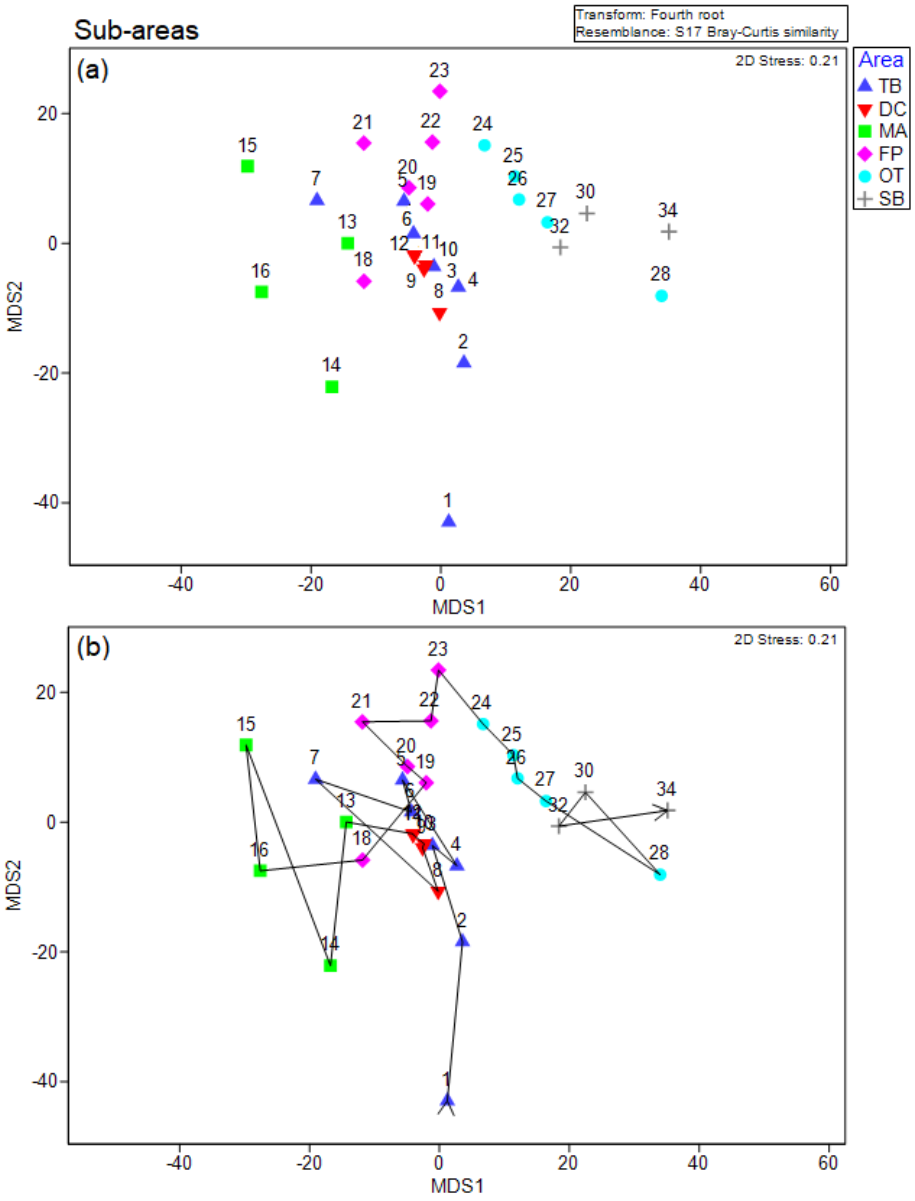


Figure 26. RB 2016: mMDS ordination of distances among centroids for (a) *a priori* sub-areas within areas, and (b) same plot overlain with trajectory of change in species assemblages between sub-areas 1 to 34. Ordination based on Bray-Curtis similarity (4th root transformed abundance). Samples are colour-coded by area and labelled by sub-area. Optimum solution in 3D with stress = 0.13.

3.3.3 Comparison to MonRoeb16

Analyses were conducted to assess how representative the sampling at Fall Point and One Tree, under the MonRoeb monthly monitoring program, was to the fauna of the whole bay sampled under the BIMs program. This was to determine if monitoring at these two locations reflected the fauna for the local area or the broader part of the bay, or the whole bay. Figure 27 presents the results from k-R cluster analysis on combined MonRoeb16 (6 cores/sample) and AnnRoeBIM-16 (pooled at 9 cores/sample) data. Sample groups were similar to those apparent from cluster analysis on AnnroeBIM16 data alone (refer section 3.3.2 Figure 20). Samples from the inner, more species-rich Fall Point MonRoeb station, FP-A, grouped with samples from median inundation zones in the TB, DC and FP areas, while samples from the outer Fall Point MonRoeb station, FP-B, grouped with inner stations in the samples from lower inundation zones in the TB, DC and FP areas. Samples from inner One Tree MonRoeb station, OT, grouped with samples from Southern Beach.

Bray-Curtis similarities underpinning these cluster groups are depicted as bubble plots in Figure 28, where the larger the bubble, the greater the average percent similarity between MonRoeb and AnnRoeBIM samples. Again, a large degree of spatial variation is obvious in the data.

This analysis indicates that the One Tree MonRoeb samples are most similar to assemblage composition for the Southern Beaches, whereas the Fall Point samples are more similar to the eastern part of RB, but with the outer station aligned with higher inundation areas, and the inner station more similar to lower inundation samples. These relationships hold at the higher classification level, but the Bray-Curtis similarities show that there is high between site variation in similarities, and the relative similarities of the MonRoeb sites are relatively low and very variable when compared to adjacent AnnRoeBIM samples.

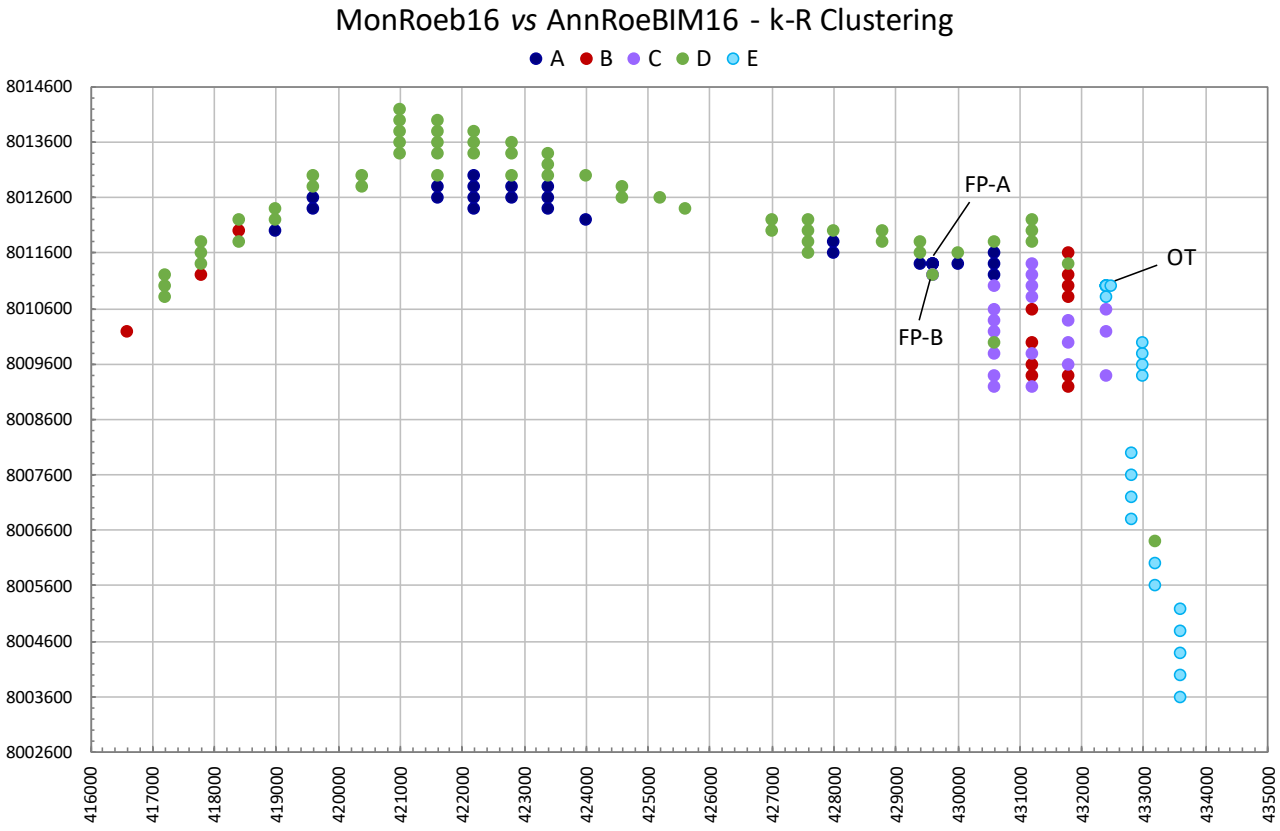


Figure 27. Station groupings (A, B, C, D, E) determined from k-R clustering (non-hierarchical) on species assemblages (4th root transformed abundance), based on Bray-Curtis similarity for un-pooled MonRoeb16 samples (6 cores per sample) and pooled AnnReoBIM16 samples (9 cores per sample). MonRoeb16 stations are indicated as FP-A, FP-B and OT.

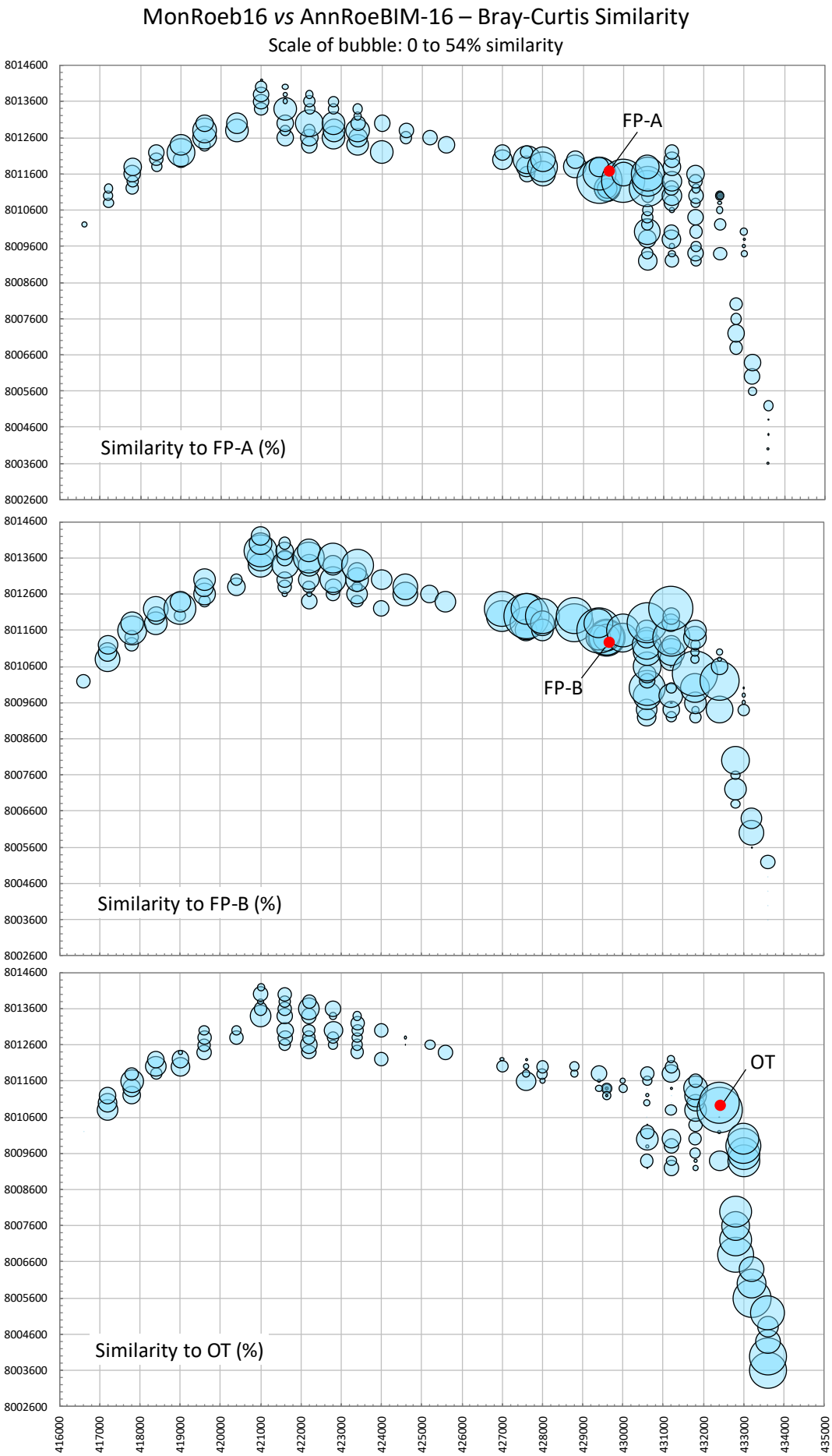


Figure 28. Average percent pairwise similarity (Bray-Curtis) of AnnRoeBIM-16 stations (9 cores/sample) to each MonRoeb16 station (6 cores/sample); FP-A, FP-B and OT.

3.4 Temporal Variability in the Biota

3.4.1 Univariate Metrics

Seasonality and between-year changes in the fauna were investigated and reported by Compton (2017) using the MonRoeb data, but between-survey differences in the BIMs data has not been assessed. Year-to-year changes in average (± 95% CI) values for species metrics for each BIMs area are shown in Figure 29. There were significant between-year differences in species richness and abundance for most areas (two-factor ANOVA, Year x Sub-area, Table 8), but no consistent significant upward or downward trends either within or among areas. The most notable change was a decrease in species richness and abundance across all areas in June 2002, followed by recovery in subsequent years. There was a concomitant change in Shannon-Weiner diversity across all areas in 2002, though this was relatively weak for OT. It was likely that the comparatively low species richness, abundance and diversity in 2002 was a direct response to Cyclone Rosita which crossed the bay in April 2000.

Significant sub-area effects were also detected within some areas (Table 8), and appeared to reflect longitudinal gradients around the bay. For example, pairwise tests (not shown) for sub-areas within TB, indicated significantly lower species richness and diversity in sub-area 2 toward the west, compared to other sub-areas, while sub-area 7 on the east, had significantly higher species richness and diversity. These gradients appeared consistent across years, based on the non-significant interaction terms (Year x Sub-area, $p > 0.01$). Similarly, pairwise tests for area FP, showed significantly higher species richness in sub-areas 22 and 23 toward the eastern side of FP, compared to most other FP sub-areas. In contrast, for area OT, species richness, abundance and diversity showed a decreasing gradient from west (sub-area 24) to east (sub-area 28), with an associated increase in evenness.

Table 8. Two-factor ANOVA testing for significant ($p < 0.05$) Year and Sub-area effects on species-level diversity indices (after pooling) for each RB area. *S* = species richness; *N* = total abundance (log_{10} transformed); *J'* = evenness (log_{10} transformed), H' (log_e) = Shannon-Weiner diversity. Significant *p* values are shaded blue for clarity.

Area	Source	Variable	ANOVA (main effects)			
			df	MS	F	*p*
TB	Year	*S*	3	209.298	7.768	0.001
		N	3	0.152	5.197	0.008
		J'	3	0.001	2.561	0.082
		H' (log_e)	3	0.073	1.117	0.364
	Sub-area	*S*	6	192.836	7.157	<0.001
		N	6	0.054	1.837	0.140
		J'	6	0.000	0.934	0.492
		H' (log_e)	6	0.294	4.469	0.005
	Year x Sub-area	*S*	9	34.089	1.265	0.311
		N	9	0.050	1.702	0.151
		J'	9	0.001	1.906	0.107
		H' (log_e)	9	0.032	0.488	0.866
	Residual	*S*	21	26.944	--	--
		N	21	0.029	--	--
		J'	21	0.001	--	--
		H' (log_e)	21	0.066	--	--
	Total df (each metric) = 40					
DC	Year	*S*	4	389.593	14.358	<0.001
		N	4	0.218	3.741	0.007
		J'	4	0.065	7.517	<0.001
		H' (log_e)	4	2.709	14.546	<0.001
	Sub-area	*S*	4	73.434	2.706	0.033
		N	4	0.132	2.273	0.065
		J'	4	0.004	0.463	0.762
		H' (log_e)	4	0.195	1.044	0.387

Area	Source	Variable	ANOVA (main effects)			
			df	MS	F	*p*
	Year x Sub-area	*S*	12	62.220	2.293	0.012
		N	12	0.111	1.901	0.041
		J'	12	0.006	0.738	0.712
		H' (log$_e$)	12	0.175	0.942	0.508
	Residual	*S*	120	27.134	--	--
		N	120	0.058	--	--
		J'	120	0.009	--	--
		H' (log$_e$)	120	0.186	--	--
	Total df (each metric) = 141					
MA	Year	*S*	4	415.006	13.340	<0.001
		N	4	0.173	1.506	0.215
		J'	4	0.011	0.290	0.883
		H' (log$_e$)	4	0.711	2.021	0.106
	Sub-area	*S*	4	281.791	9.058	<0.001
		N	4	0.040	0.351	0.842
		J'	4	0.035	0.924	0.458
		H' (log$_e$)	4	0.765	2.176	0.086
	Year x Sub-area	*S*	9	146.673	4.715	<0.001
		N	9	0.195	1.695	0.116
		J'	9	0.062	1.626	0.135
		H' (log$_e$)	9	0.683	1.943	0.068
	Residual	*S*	48	31.111	--	--
		N	48	0.115	--	--
		J'	48	0.038	--	--
		H' (log$_e$)	48	0.352	--	--
	Total df (each metric) = 66					
FP	Year	*S*	4	116.953	5.217	0.002
		N	4	0.298	2.914	0.032
		J'	4	0.113	4.814	0.003
		H' (log$_e$)	4	2.280	9.341	<0.001
	Sub-area	*S*	5	68.689	3.064	0.018
		N	5	0.125	1.224	0.314
		J'	5	0.013	0.569	0.723
		H' (log$_e$)	5	0.160	0.656	0.658
	Year x Sub-area	*S*	12	25.368	1.132	0.360
		N	12	0.037	0.364	0.970
		J'	12	0.005	0.214	0.997
		H' (log$_e$)	12	0.113	0.464	0.925
	Residual	*S*	45	22.417	--	--
		N	45	0.102	--	--
		J'	45	0.023	--	--
		H' (log$_e$)	45	0.244	--	--
	Total df (each metric) = 67					
OT	Year	*S*	4	70.515	2.507	0.045
		N	4	0.654	8.616	<0.001
		J'	4	0.023	2.139	0.079
		H' (log$_e$)	4	0.416	2.175	0.075
	Sub-area	*S*	4	443.557	15.768	<0.001
		N	4	1.314	17.310	<0.001
		J'	4	0.062	5.886	<0.001
		H' (log$_e$)	4	0.734	3.839	0.005
	Year x Sub-area	*S*	12	25.837	0.918	0.530
		N	12	0.063	0.831	0.618
		J'	12	0.005	0.489	0.919
		H' (log$_e$)	12	0.305	1.594	0.100
	Error	*S*	140	28.130	--	--
		N	140	0.076	--	--
		J'	140	0.011	--	--

Area	Source	Variable	ANOVA (main effects)			
			df	MS	F	*p*
		H' (log$_e$)	140	0.191	--	--
	Total df (each metric) = 161					
SB	Year	*S*	3	209.298	7.768	0.001
		N	3	0.152	5.197	0.008
		J'	3	0.001	2.561	0.082
		H' (log$_e$)	3	0.073	1.117	0.364
	Sub-area	*S*	6	192.836	7.157	<0.001
		N	6	0.054	1.837	0.140
		J'	6	<0.001	0.934	0.492
		H' (log$_e$)	6	0.294	4.469	0.005
	Year x Sub-area	*S*	9	34.089	1.265	0.311
		N	9	0.050	1.702	0.151
		J'	9	0.001	1.906	0.107
		H' (log$_e$)	9	0.032	0.488	0.866
	Residual	*S*	21	26.944	--	--
		N	21	0.029	--	--
		J'	21	0.001	--	--
		H' (log$_e$)	21	0.066	--	--
	Total df (each metric) = 40					

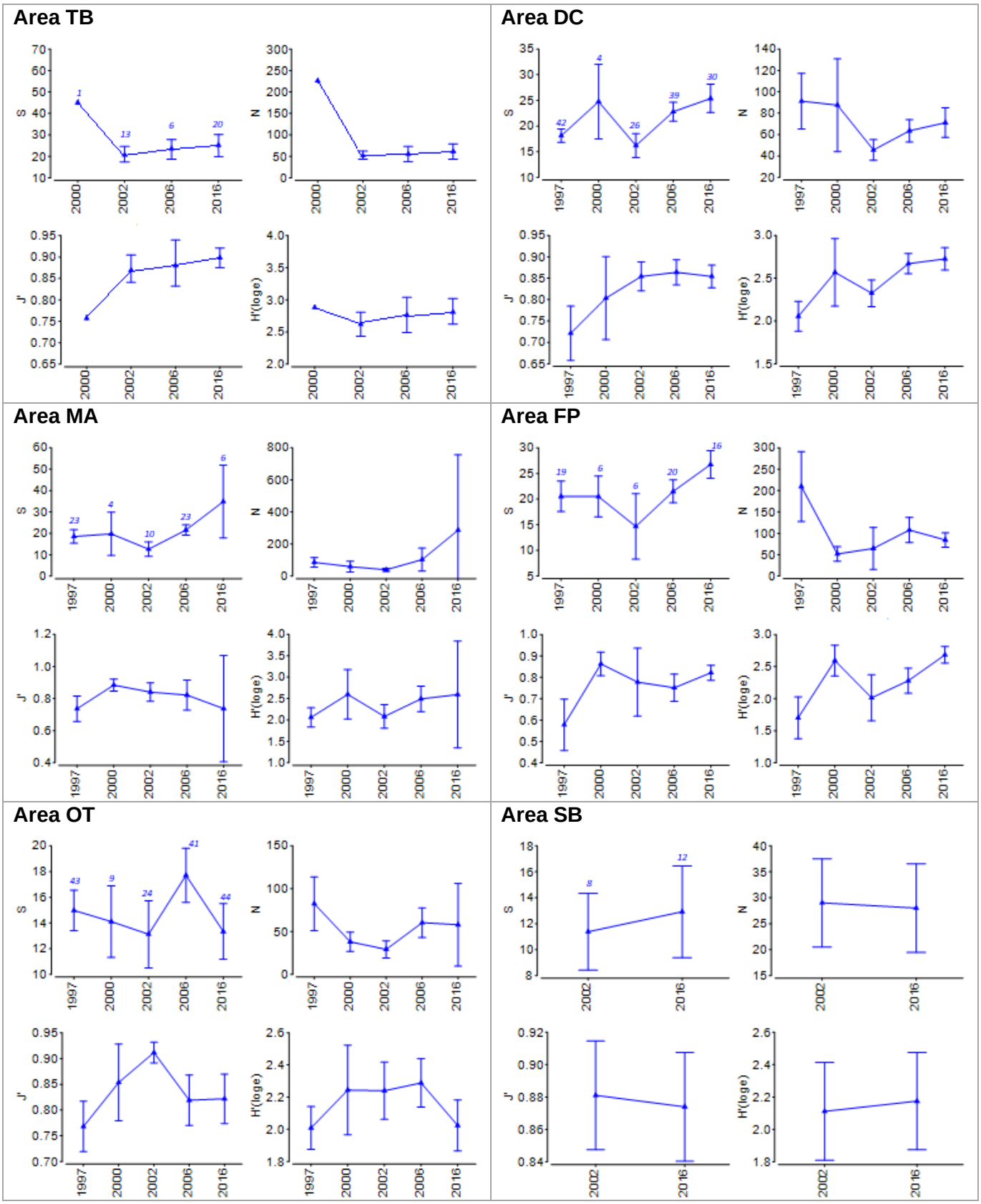


Figure 29. Between-year comparison of diversity indices (average ± 95% CI) for species-level data (after pooling) for each RBB area. *S* = total number of species; *N* = total number of individuals; *H'* $\log_e$ = Shannon-Wiener diversity; *J'* = Pielou's evenness index. Number of samples after pooling is provided in blue on the species richness plots for each area.

3.4.2 Multivariate Metrics

Within each area, there were significant temporal differences in species assemblages (PERMANOVA, Table 9), and pairwise comparisons showed most years were significantly different to each other ($p < 0.001$). The exception was 2000, where few samples were available for testing after pooling within areas DC, TB, MA, FP and OT, and hence results for 2000 are not conclusive. There were also significant differences among sub-areas within TB, MA and SB that were consistent across years (Table 9).

Table 9. Two-factor PERMANOVA testing for significant ($p < 0.05$) Year and Sub-Area effects on RB species assemblages (4^{th} root transformed abundance) within each area (TB, DC, MA, FP, OT, SB).

Area	Source	PERMANOVA (main effects)				Estimated Components of variation	
		df	MS	Pseudo-F	*p*	Var.	SD
DC	Year	4	21913	10.003	0.001	775.5	27.8
	Sub-Area	4	2150	0.981	0.520	-1.6	-1.3
	Year x Sub-Area	12	2352	1.073	0.217	24.1	4.9
	Residual	120	2191	--	--	2190.7	46.8
	Total	140	--	--	--		
TB	Year	3	6036	2.914	0.001	578.2	24.0
	Sub-Area	6	2841	1.371	0.018	173.5	13.2
	Year x Sub-Area	9	1926	0.930	0.712	-77.0	-8.8
	Residual	21	2071	--	--	2071.4	45.5
	Total	39	--	--	--		
MA	Year	4	9126	4.847	0.001	761.3	27.6
	Sub-Area	4	3189	1.694	0.002	143.2	12.0
	Year x Sub-Area	9	1837	0.976	0.565	-14.1	-3.7
	Residual	48	1883	--	--	1882.7	43.4
	Total	65	--	--	--		
FP	Year	4	11081	6.164	0.001	825.3	28.7
	Sub-Area	5	2984	1.660	0.001	122.0	11.0
	Year x Sub-Area	12	2106	1.171	0.034	105.0	10.2
	Residual	45	1798	--	--	1797.8	42.4
	Total	66	--	--	--		
OT	Year	4	21235	9.914	0.001	761.4	27.6
	Sub-Area	4	13620	6.359	0.001	442.5	21.0
	Year x Sub-Area	12	3916	1.828	0.001	249.4	15.8
	Residual	140	2142	--	--	2141.9	46.3
	Total	160	--	--	--		
SB	Year	1	6940	3.888	0.001	677.7	26.0
	Sub-Area	2	3461	1.939	0.007	307.0	17.5
	Year x Sub-Area	2	2151	1.205	0.245	133.9	11.6
	Residual	14	1785	--	--	1784.9	42.2
	Total	19	--	--	--		

The plots of mMDS ordinations of distances among centroids for years showed that within areas DC, TB, MA and OT, samples from 2002 and 2016 also tended to separate from samples from other years (Figure 30). In general, species assemblages in 2002 and 2016 appeared to be slightly less similar to those recorded in the initial sampling year (taken here to represent baseline condition), particularly when compared to 2006 assemblages (Figure 30). It is not known if this was in part due to the lower number of samples collected in 2000, resulting in a higher similarity to baseline for that year compared to 2002 and 2016. It was expected that lower similarity to baseline would have been recorded in 2002, when lower species richness, abundance and diversity were recorded, likely in response to Cyclone Rosita in April 2000. While the increased similarity in 2006 may well reflect on-going recovery post-cyclone, it is difficult to explain the decrease in 2016 as a response to any specific event without data for intervening years to

ascertain trends. However, the year-to-year changes detected within each area were typically small (≤ 10%) and as such, are likely well within the range of stochastic variability unrelated to specific events such as cyclones. The estimated components of variation (Table 8) suggested only about one third to one half of the total variation was attributable to specific year effects (as can be seen by the comparatively large Residuals). The largest temporal change was recorded at TB, with an 18% decline in similarity to baseline between 2006 and 2016 (Figure 31). Again, this was due to small changes in abundance of a large number of species (~50), each contributing < 3% to the total variation between years. The absence of quantitative data on potential driving factors, such as changes in water temperature, coverage of algal (*Lyngbya*) blooms, nutrient levels, other potential contaminants etc makes it impossible to attribute the observed changes to an 'impact' per se, but such an influence should not be discounted. As above, the changes may be natural stochastic changes, but spatially concentrated changes, such as declines at TB where there are known pressures, should be tracked over the future.

For all areas, within-year similarity among samples was lowest in 2016, most notably at OT with a minimum of 19.5% in 2016 compared to a maximum (not including 2000) of 36.8% in 1997 (Figure 31).

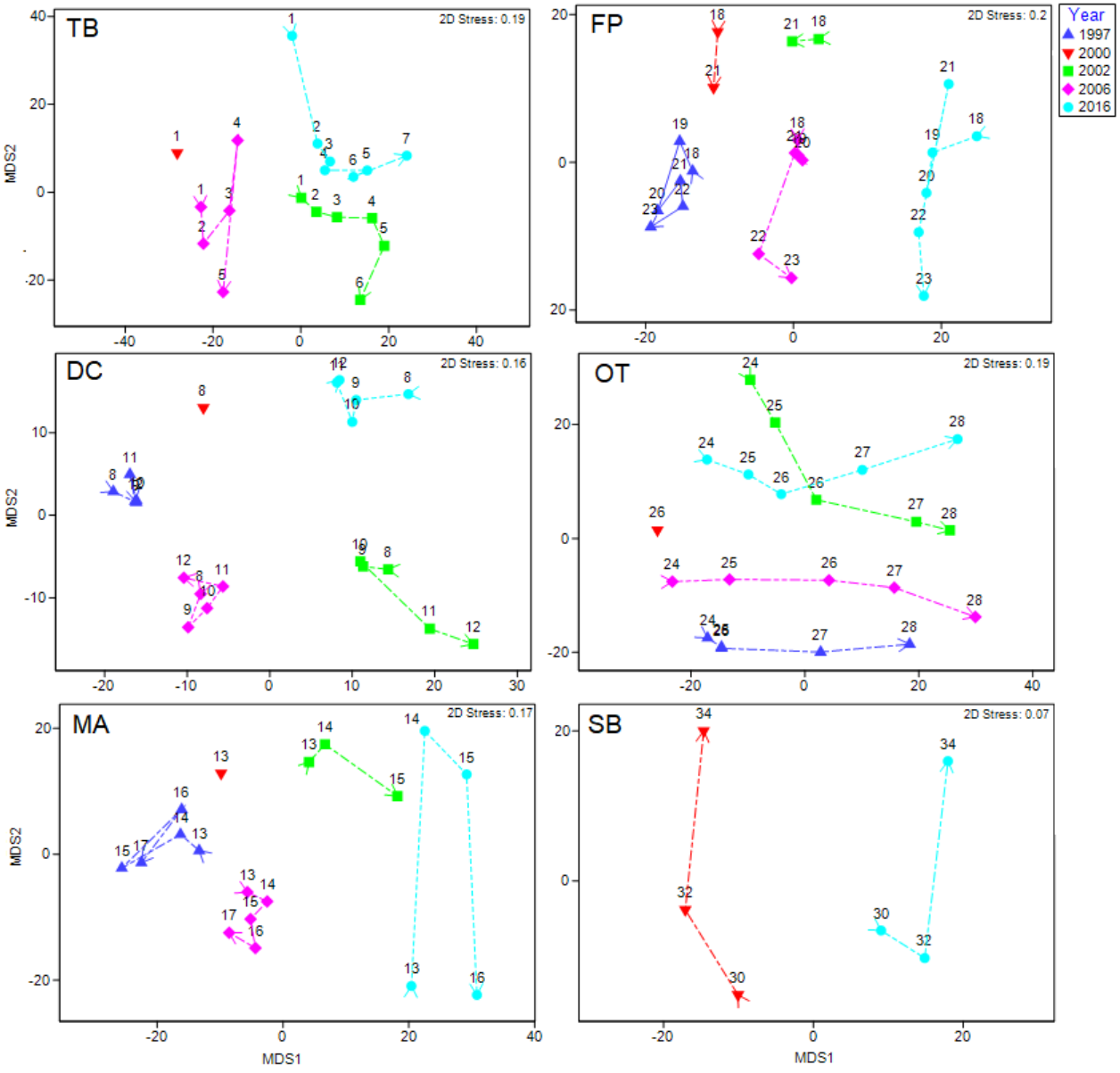
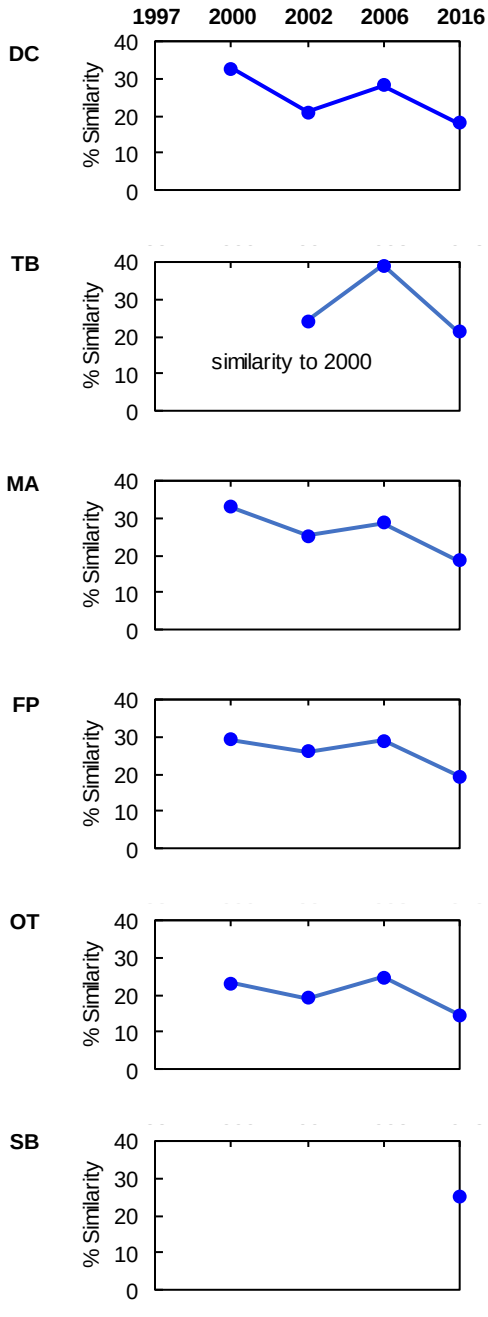


Figure 30. RB 1997-2016: mMDS ordinations of distances among sub-area centroids for each *a priori* area, overlain with trajectory of change in species assemblages between sub-areas. Ordinations based on Bray-Curtis similarity (4th root transformed abundance). Samples are colour-coded by year and labelled by sub-area. Optimum solutions in 3D with stress for TB = 0.12, DC = 0.09, MA = 0.11, FP = 0.12, OT = 0.11, and SB = 0.03.



		1997	2000	2002	2006	2016
DC	1997	35.0				
	2000	32.8	53.0			
	2002	21.1	28.9	34.5		
	2006	28.3	36.0	24.3	38.1	
	2016	18.2	28.0	22.1	20.7	28.5
TB	1997	--				
	2000	--	0.0			
	2002	--	24.2	38.1		
	2006	--	39.1	28.2	47.2	
	2016	--	21.4	27.2	24.4	28.4
MA	1997	36.1				
	2000	33.1	49.7			
	2002	25.2	31.8	40.5		
	2006	28.7	30.2	26.4	39.4	
	2016	18.5	24.7	25.0	22.3	31.3
FP	1997	39.3				
	2000	29.2	41.4			
	2002	26.1	26.1	36.8		
	2006	28.9	32.6	29.2	41.2	
	2016	19.4	21.6	22.2	25.7	33.3
OT	1997	36.8				
	2000	23.2	44.0			
	2002	19.2	18.5	25.7		
	2006	24.8	23.2	19.1	30.2	
	2016	14.5	14.8	15.2	16.0	19.5
SB	1997	--				
	2000	--	--			
	2002	--	--	41.9		
	2006	--	--	--	--	
	2016	--	--	25.3	--	31.5

Figure 31. Plots of change in average similarity (Bray-Curtis) to 'baseline' (*i.e*. earliest year sampled), together with average pairwise percent similarity between/within years for each RBB area. Note, TB was not sampled in 1997, and SB was not sampled in 1997, 2000 or 2006.

3.5 Detectable Change – Power Analysis

Power analysis was used to calculate how many samples would be needed to detect a specified change (*i.e*. effect size) in univariate metrics, and to determine if temporal change (indicated from temporal analyses; section 3.4) was greater than detectable change indicated by power analysis.

For this analysis, MonRoeb-16 data for stations FP-A, FP-B and OT-A were used, as these stations were sampled monthly, thereby i) affording larger datasets from single locations to test, and ii) avoiding potential confounding effects of environmental influences on species data when pooling samples over larger distances, as would be necessary for BIMs data. Only species richness and abundance data were tested, as the variance within these datasets was either similar to, or greater than, the variance within diversity (Shannon-Weiner) and evenness data, and therefore captured the variance encountered within all diversity measures (*i.e*. power is related to variance, and so statistical power for less variable datasets would be better than that calculated for the richness and abundance data).

Results are summarised in Table 10 and suggest that under the current method for MonRoeb (6 cores per station), a minimum of 33 replicate samples would be required to detect a 10% decline in average species richness at FP-A, but 109 replicates would be required to detect a similar decline in average species richness at OT-A. For abundance, a minimum of 37 replicates would be required to detect a 10% decline at FP-B, but 144 replicates would be required to detect a similar decline at OT-A. The differences in the estimated number of replicates required, reflects the variance in the underlying datasets.

Table 10. Number of samples required to detect a specific change (10%, 20%, 30%, 40%, 50%) in average species richness and abundance at fall Point and One Tree stations, based on power analysis on 2016 data.

	MonRoeb16					No. of samples required for specific effect size				
Species Metric	**Station**	**No. cores**	**No. reps**	**Mean**	**SD**	**10%Δ**	**20%Δ**	**30%Δ**	**40%Δ**	**50%Δ**
Species richness	FP-A	**6**	4	26.0	4.24	33	9	4	3	2
	FP-B	**6**	4	15.0	2.60	38	10	5	3	2
	OT-A	**6**	8	9.4	2.77	109	28	13	7	5
	FP-A	**12**	4	35.3	2.99	9	3	1	1	1
	FP-B	**12**	4	20.5	2.65	21	6	3	2	1
	OT-A	**12**	8	14.9	2.23	28	7	4	2	2
	FP-A	**18**	4	41.8	2.22	12	9	7	5	4
	FP-B	**18**	4	24.8	2.22	10	3	2	1	1
	OT-A	**18**	8	20.1	2.03	13	4	2	2	1
	OT-A	**24**	8	26.1	1.96	7	2	1	1	1
Total abundance	FP-A	**6**	4	186.8	59.89	128	32	15	8	6
	FP-B	**6**	4	42.5	7.33	37	10	5	3	2
	OT-A	**6**	8	17.4	5.93	144	36	16	9	6
	FP-A	**12**	4	341.3	55.70	33	9	4	3	2
	FP-B	**12**	4	84.8	10.08	18	5	2	2	1
	OT-A	**12**	8	32.6	6.70	53	14	6	4	3
	FP-A	**18**	4	554.3	61.59	16	4	2	1	1
	FP-B	**18**	4	124.5	7.59	5	2	1	1	1
	OT-A	**18**	8	50.8	5.99	18	5	2	2	1
	OT-A	**24**	8	69.8	6.25	10	3	2	2	1

Far fewer samples however, would be required to detect a 20% change in these metrics, *e.g*. 9 replicates for species richness at FP-A and 10 replicates for species abundance at FP-B. It was considered that the ability to detect a 10% change, although highly desirable in impact assessment, is a relatively stringent assessment criterion for detecting future impacts and would also require excessively high replication. A 20% change may therefore be a more acceptable assessment criterion, or a level of replication that is logistically, and financially acceptable (*i.e*. 20 samples) would provide a level of statistical power better than 20% effect size, but not as good as an optimum effect size of 10%. Similarly, the results show that as the number of cores per station is increased, the number of replicates needed to detect a specific effect size, decreases. For example, increasing from 6 to 12 cores per station, the estimated number of replicates required to detect a 20% decline in species richness ranged from 3 to 7, and for species abundance, from 5 to 14. Thereby taking larger samples (12 cores instead of 6 cores per sample) reduces inter-sample variability, and improves ability to detect significant changes should they occur (*i.e*. better statistical power).

Between-year variability in average species richness and abundance in MonRoeb data was comparatively large, even when comparing the same month across year. Plots for June and October data for 1996 to 2016 are provided in Figures 32 and 33 and show average values for samples (6 cores per sample) typically varied by more than 20% each year. For June data, greatest change in species richness between consecutive years was 67% (FP-B, 2006 to 2007), and greatest change in abundance was 74% (FP-B, 2013 to 2014) (Figure 32). For October data, greatest change in species richness between consecutive years was 60% (FP-B, 1996 to 1997), and greatest change in abundance was 50% (OT-A, 1996 to 1997) (Figure 33). It is of course unknown if this reflects natural variability in the fauna, or a response to unknown anthropogenic impacts. Either way, the between month/year changes are greater than the effect size that the design would have, based on power analysis, and therefore, the sampling design would detect these as significant changes.

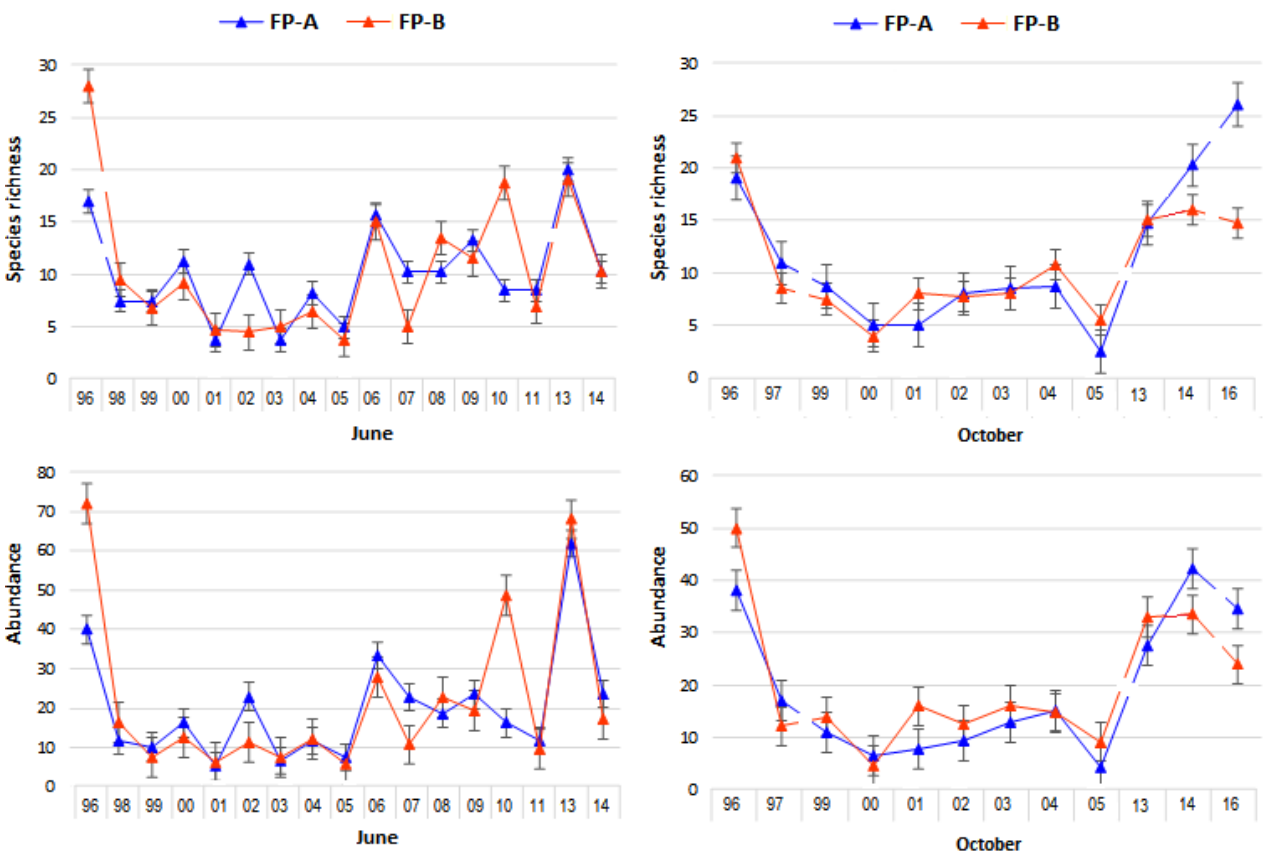


Figure 32. MonRoeb, Fall Point: temporal variability in average (± SE) species richness (top) and abundance (bottom) at each station, based on available June (1996 - 2014) and October (1996 - 2016) data for each year sampled. Note, from 1999 to 2005 (inclusive) not all polychaete families were counted or identified (refer section 2.1.2).

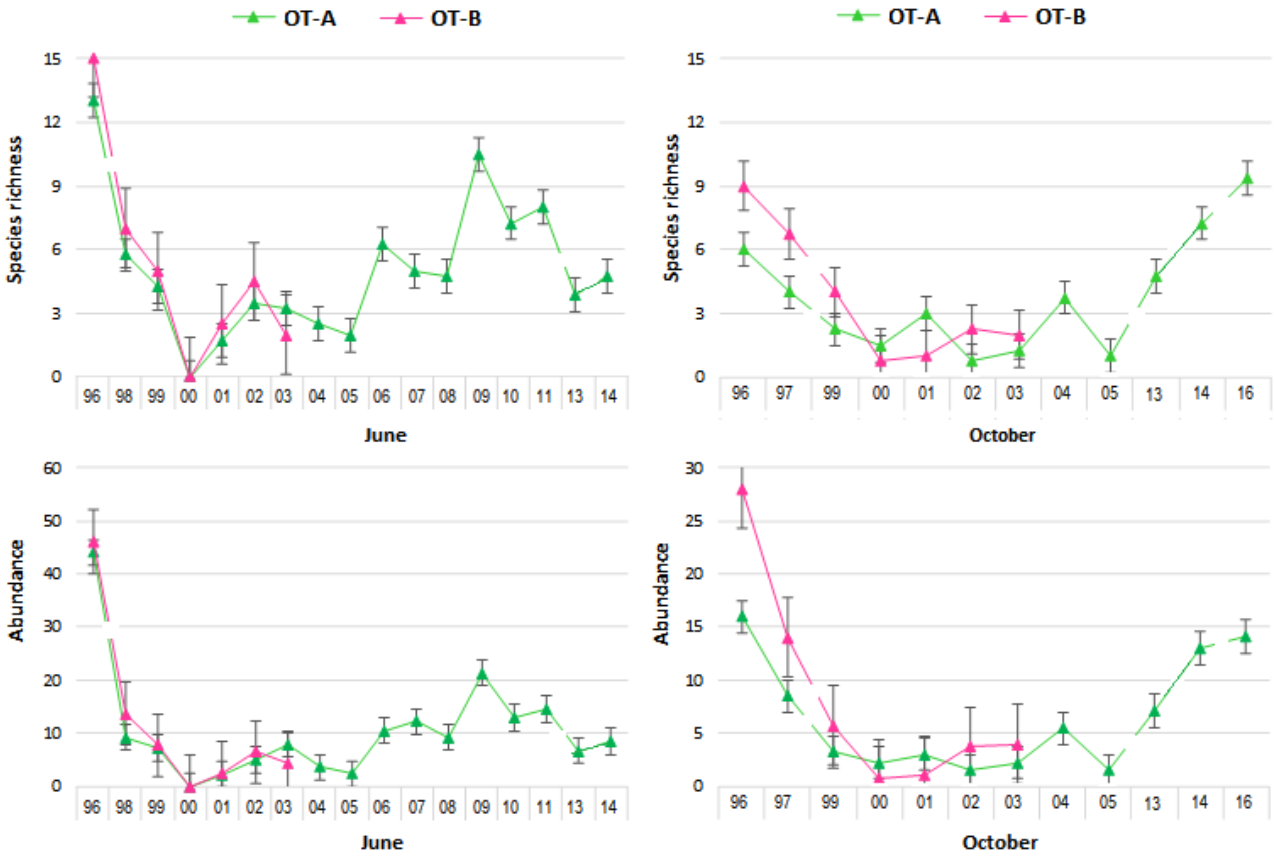


Figure 33. MonRoeb, One Tree: temporal variability in average (± SE) species richness (top) and abundance (bottom) at each station, based on June and October data for each year. Note, from 1999 to 2005 (inclusive) not all polychaete families were counted or identified (refer section 2.1.2).

For comparison, percent change between years is summarised for pooled BIM samples (9 cores per sample) in Figure 34. Similar to MonRoeb data, average values for species metrics within each area TB, DC, MA, FP, OT and SB, typically varied by more than 20% each year. Assuming power to detect temporal change would be similar for the AnnaRoeBIM program if 9 cores per site were collected instead of 3 cores, detectable change would therefore appear to be less than temporal variability (*i.e*. the design would have sufficient statistical power to detect these changes). However, greatest change was typically recorded between 2000 and 2002, associated with strong declines in species richness (max. 54% at TB) and abundance (max. 77% at TB), and between 2002 and 2006, associated with strong increases in species richness (max. 47% at FP) and abundance (160% at MA) (Figure 34). Change in metrics between other sampling years, *i.e*. between 1997 and 2000, and between 2006 and 2016, tended to be much lower; typically ≤ 25%. The differences in magnitude of between-year changes pre- and post-2000 were potentially due to the impact of Cyclone Rosita, and related to declines in species richness, abundance and diversity subsequently recorded in 2002, followed by recovery in 2006. Based on this power analysis, the pooling of BIMs samples to 9 cores per sample appears to be a reasonable compromise as reflected by the power analysis on MonRoeb data using 6 and 12 cores per sample. Such level of replication and sample size will detect current between-year changes, and if conducted on an annual basis, with supporting physico-chemical data, should be able to detect systematic changes in benthic fauna, and relate these changes to potential stressors/contaminants of concern.

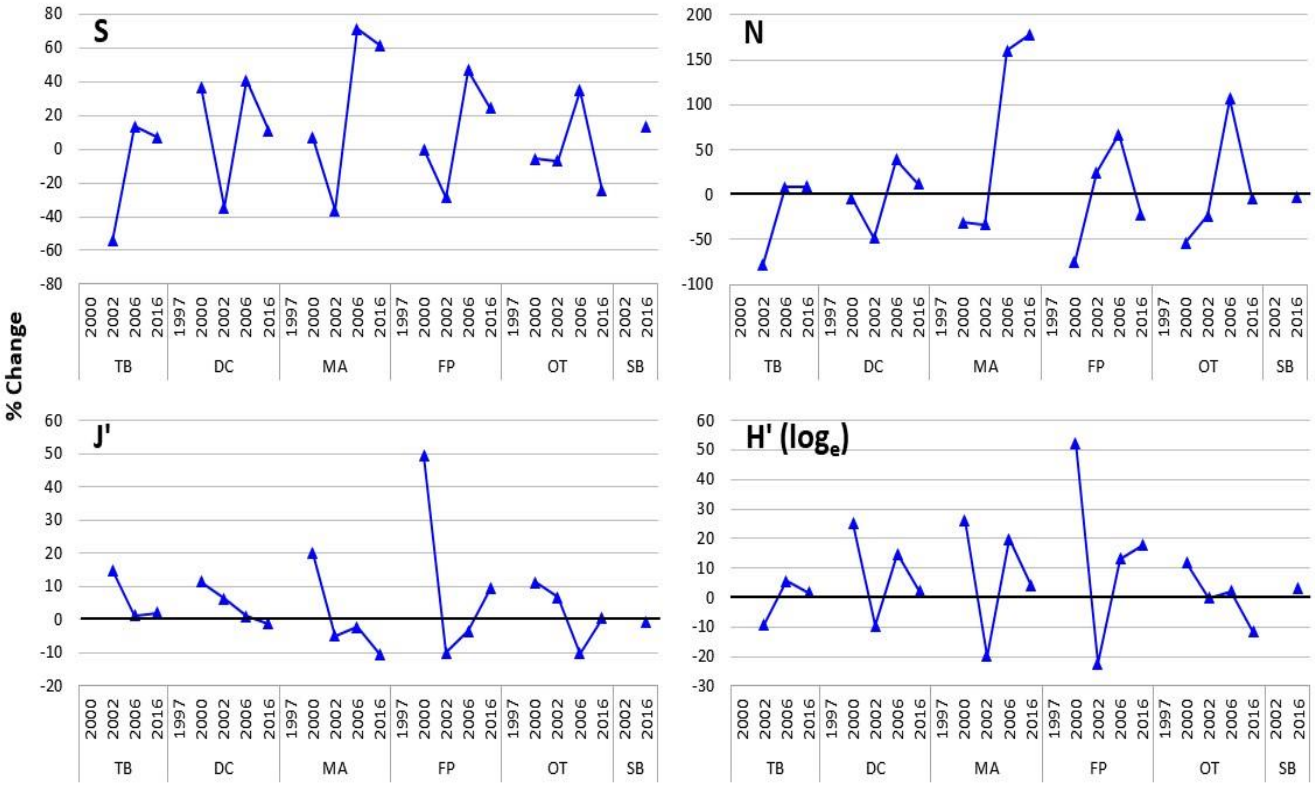


Figure 34. AnnaRoeBIM: temporal variability in average species richness (S), abundance (N), evenness and diversity (after pooling) for each area, based on all available data for each year (1997 - 2016).

4.0 SPATIO-TEMPORAL VARIABILITY WITHIN EIGHTY MILE BEACH

4.1 Dominant Environmental Factors

As discussed for RB (section 3.1), habitat characteristics such as inundation time, sediment grain size and vegetation cover are all widely reported to influence the distribution and abundance of benthic intertidal invertebrates. Inundation time, sediment penetrability and algal cover are the dominant physical variables measured as part of invertebrate mapping surveys for EMB (see Methods section 2.1.1). These were evaluated in the current analyses as potential predictors of benthic invertebrate assemblages. Unlike RB, seagrasses do not occur at EMB, as the beach is exposed to strong wave action and storm surges.

Figure 35 illustrates the qualitative inundation categories used here for EMB, though both qualitative categories and continuous data were input to analyses. As for RB, qualitative categories for EMB ranged from 0 - 30% closer to the shore, 31 - 70% in mid zones, and 71 - 100% being further out. The 71 - 100% category was well represented along the mid-section of the beach (*i.e*. area 5 and 20), but were poorly represented in areas -10, 35 and 50, and absent in areas 0 and 65.

The tidal flats along EMB are very broad, up to 4 km wide at spring low tide, narrowing to the north of area 0. The northern areas tend to be more dominated by softer muds, while the southern areas are sandier and firmer (Piersma *et al*. 2016). To date, the tidal flats in area 65 have not been comprehensively mapped as they have only been accessed during neap low tides. Nor have the flats in area 0 been fully surveyed as access is difficult due to deep muds in this area.

Penetrability was measured in 1999 and 2016, but silt content and grain size have only been analysed in 1999. Correlation and regression analyses were therefore again used to examine the relationships between penetrability in 1999 and corresponding data for silt content and median grain size (mgs), to determine if penetrability was a suitable surrogate for silt content and grain size at EMB. Penetrability was significantly correlated with silt content and, to a lesser degree mgs, though the relationships were only weakly linear (Figure 36).

Spatio-temporal variability in penetrability values between 1999 and 2016 is depicted in Figures 37, showing differences in penetrability between areas, and changes in the degree and spatial extent of penetrability between areas and between surveys. Qualitative categories for EMB sediment penetrability derived for 2016 data are illustrated in Figure 38. Of most note in 2016 was the reduction in deeper fine sediment in areas 5, 20, 35 and 50, but increase in areas -10 and 0, compared to in 1999.

Both qualitative category and continuous data were used to investigate spatial patterns in the benthic invertebrates.

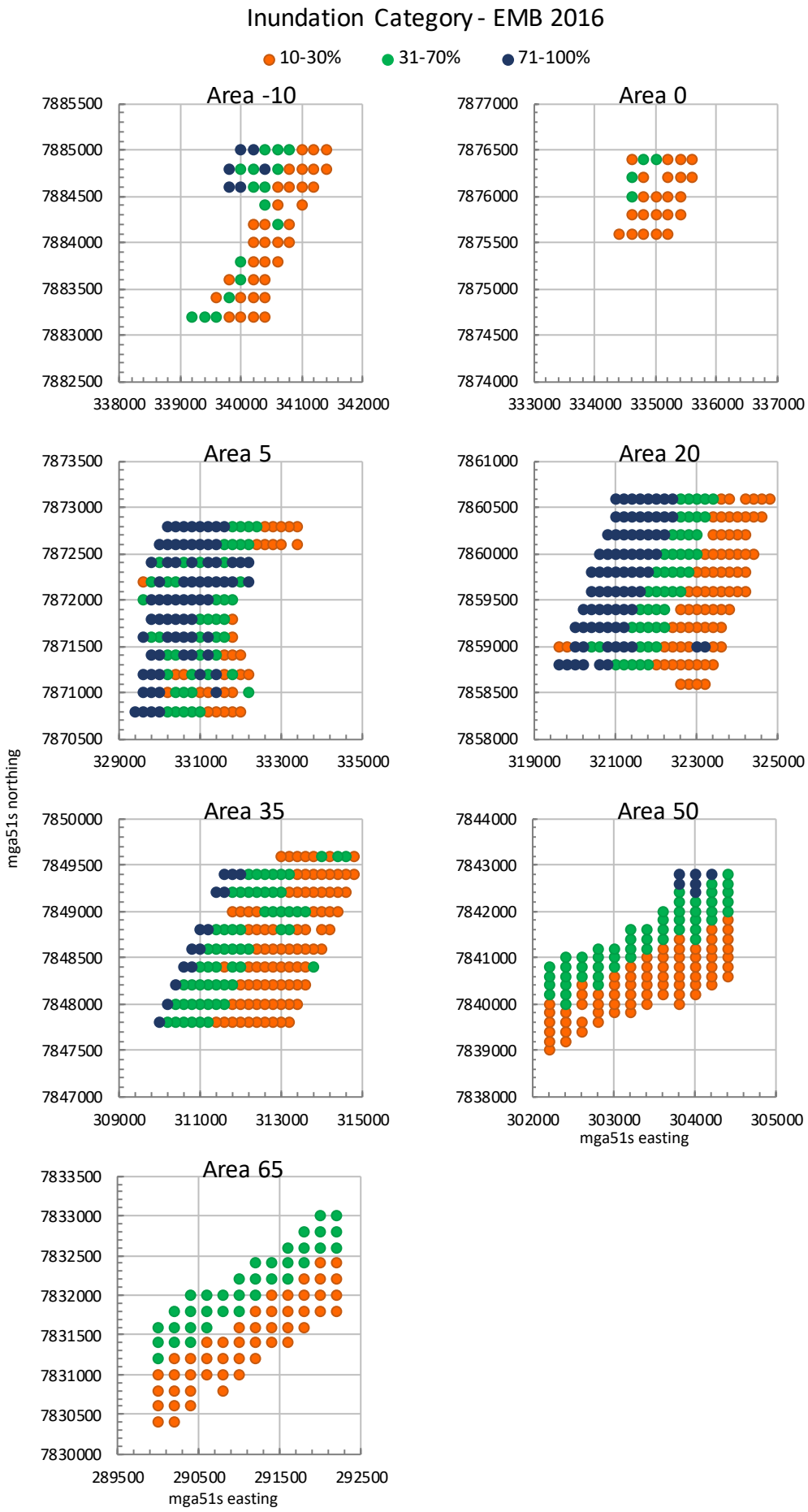


Figure 35. EMB 2016: inundation category (0-30, 31-70, 71-100%) for each station in each area.

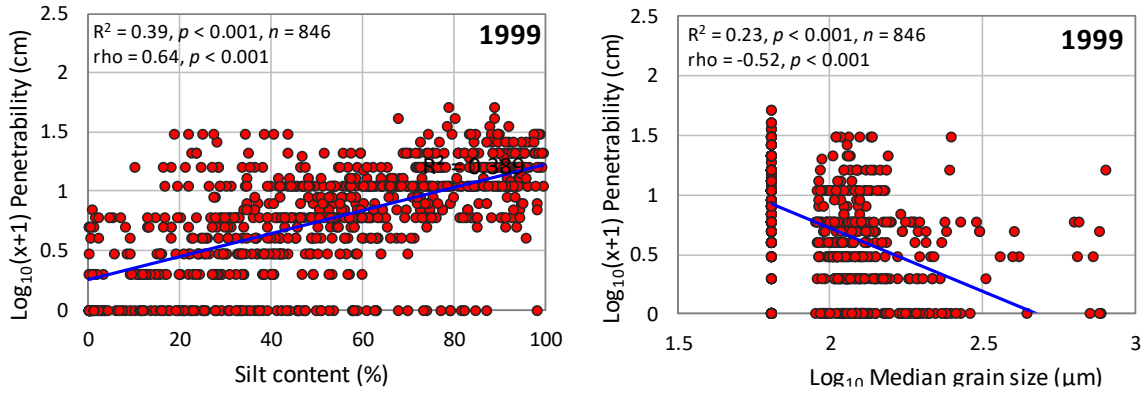


Figure 36. EMB: relationship between sediment penetrability, percent silt content (< 63 µm) and median grain size, based on 1999 data.

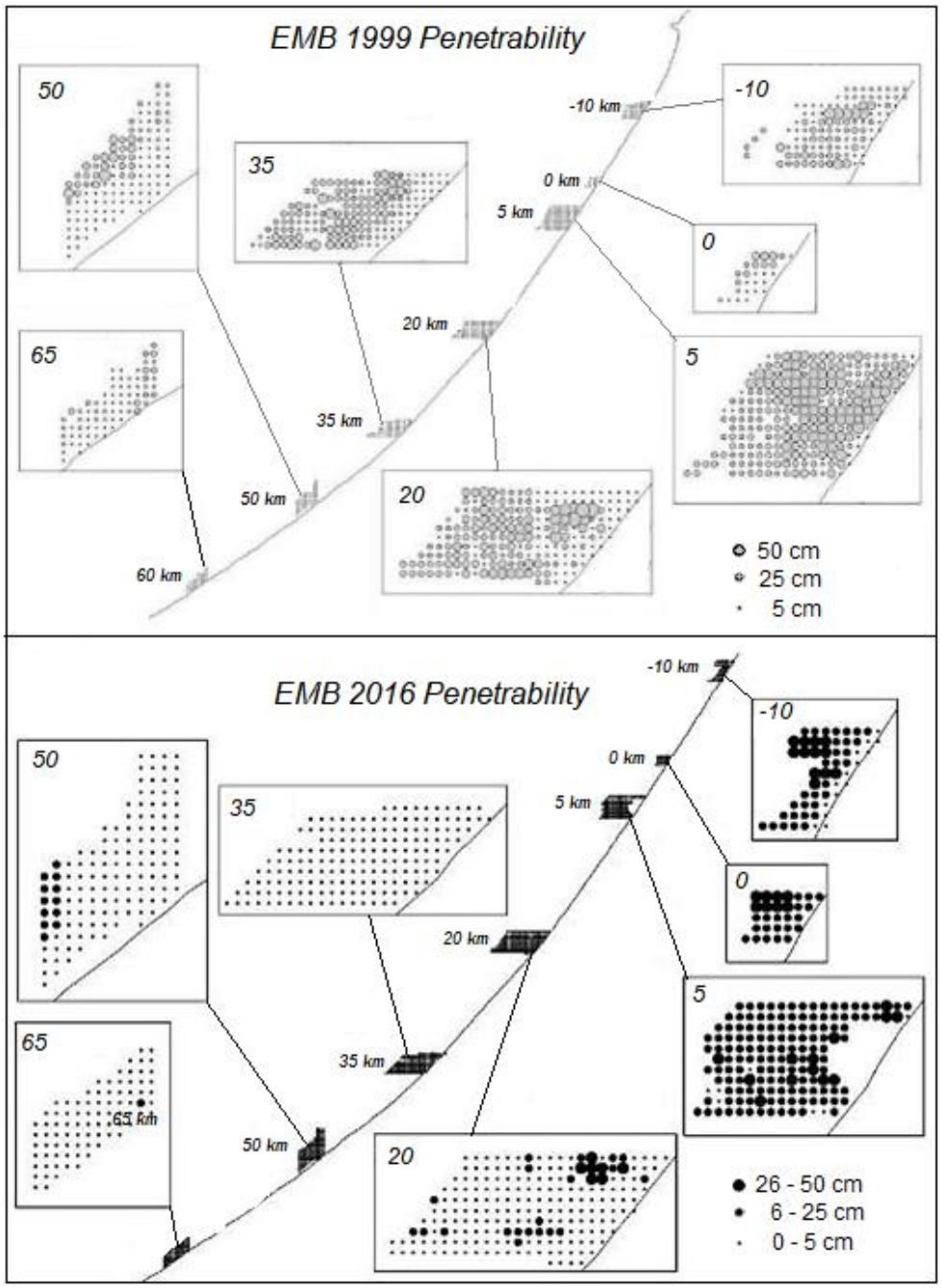


Figure 37. EMB: Penetrability (cm) on intertidal areas sampled in June 2006 (top) and October 2016 (bottom) (from Piersma *et al.* 2016).

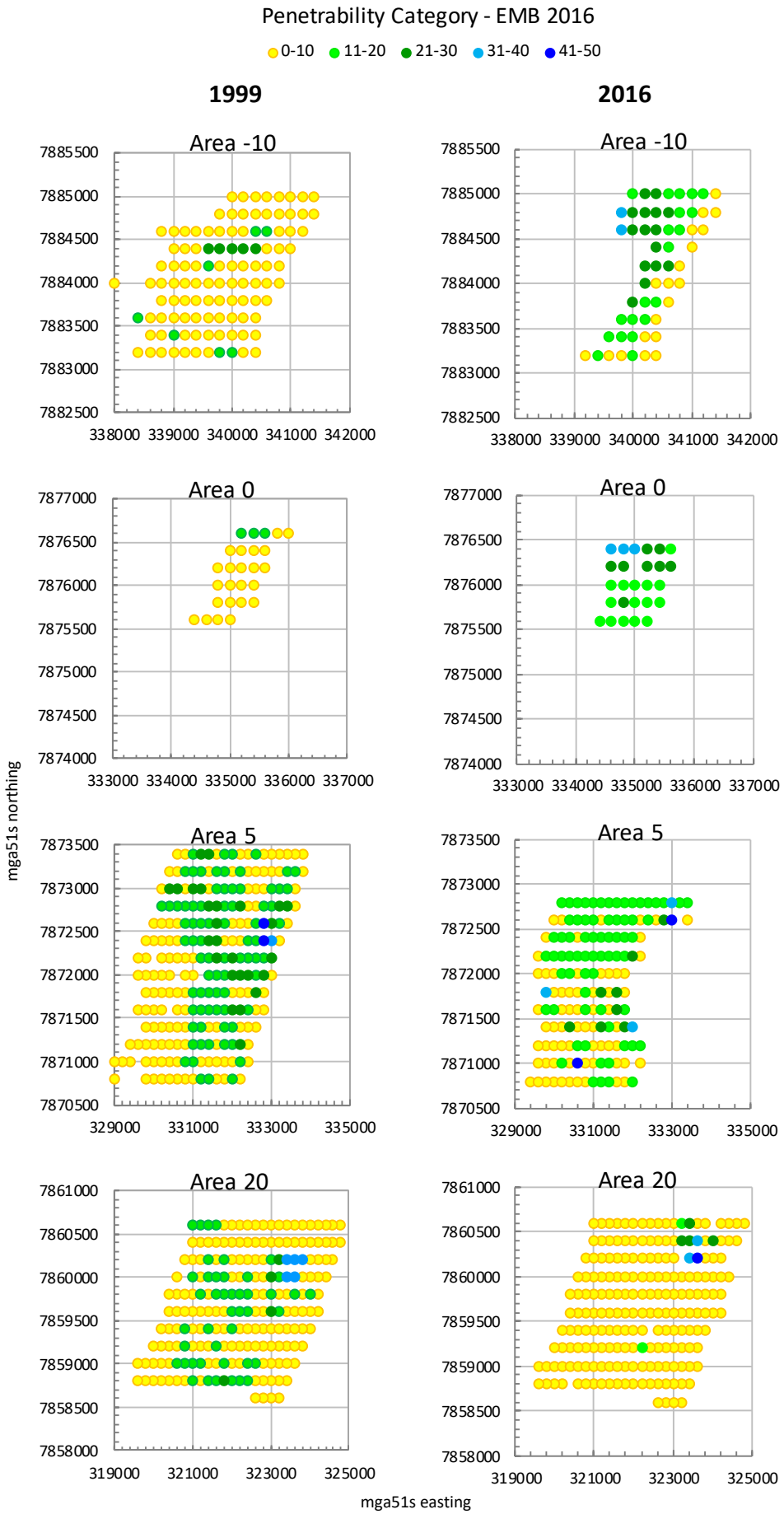


Figure 38a. EMB 1999 versus 2016: substrate penetrability category (0-10, 11-20, 21-30, 31-40, 41-50 cm) for each station in areas -10, 0, 5 and 20.

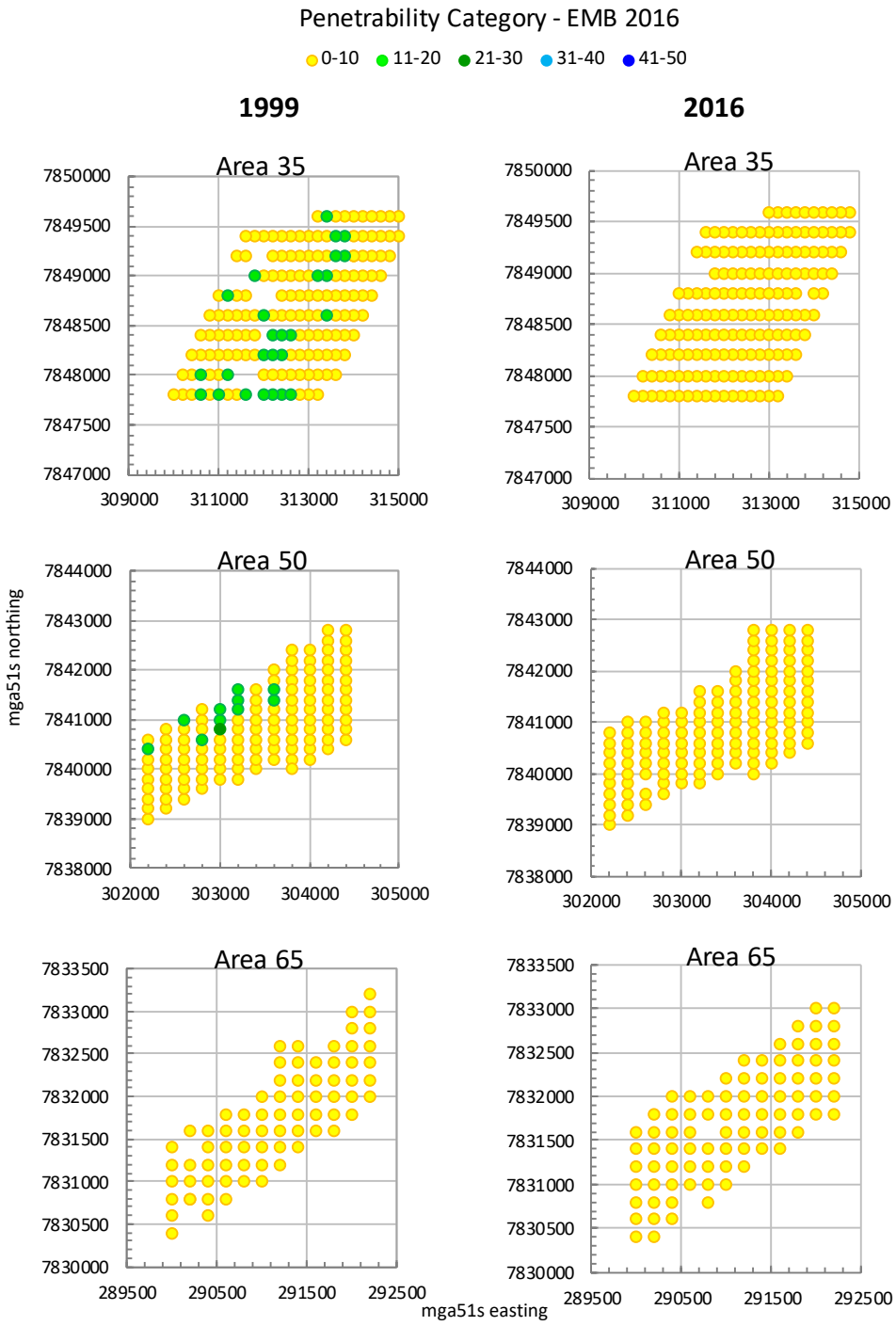


Figure 38b. EMB 1999 versus 2016: substrate penetrability category (0-10, 11-20, 21-30, 31-40, 41-50 cm) for each station in areas 35, 50 and 65.

4.2 Optimal Sampling Size (Number of Cores) for Benthic Invertebrates

The AnnaRoeBIM16 sampling for EMB recorded 20,773 benthic invertebrate specimens, representing 153 species from 15 phyla. Relative differences in the total number of species recorded for each station sampled in 2016 are illustrated in Figure 39a. An average of 5.9 species per sample was recorded, with a range in values of 0 - 16. Approximately 14% of samples (*i.e.* 115) recorded ≤ 5 species, while 2% (19) recorded zero species.

Bray-Curtis pairwise percent dissimilarity/similarity analysis on the 797 samples that contained species (19 of the 816 samples contained no fauna), showed that of the 317,206 pairwise combinations of samples, 77,109 pairs (*i.e*. 24%) shared no common species. As with RB data, this variability also suggested the sampling size (3 cores/sample, total surface area = 0.025 m^2) may be too small to be representative of the species communities present. As for RB, plots for EMB of the proportion of dissimilarity values equal to one (d = 1) and the proportion of undefined similarities (d = NaN) revealed these proportions could be minimised by pooling ≥ 3 samples (see example Figure 40). Accordingly, the skewness in the distribution of Bray-Curtis dissimilarities was greatly reduced by pooling ≥ 3 samples (Figure 41).

Therefore, to reduce the variance in the data for meaningful univariate and multivariate statistical tests, data from individual AnnaRoeBIM-16 samples were pooled, using the same rational and general approach used for RB (see section 3.2). Each pooled sample for EMB comprised 3 samples (*i.e*. 9 cores, total surface area = 0.075 m^2) grouped by *a priori* area and sub-area, as described in Methods section 2.2.1, with pooling conducted using adjacent sites along the beach to avoid pooling 'down' the beach and across inundation zones, which literature and RB analyses indicate has an influence on assemblage composition.

The effects of pooling on species richness can be seen by comparing Figure 39a with 39b, and Figure 42a with 42b. Pooling had a far greater effect in reducing the number of samples for EMB, compared to RB, because there were fewer samples which could be pooled by UTM easting within each sub-area (refer section 2.2.1), in an attempt to limit possible confounding effects of inundation. Pooling reduced the number of samples from 819 to 190, increased average number of species per sample from 5.9 to 12.7, and reduced the number of samples with few species (*i.e*. < 5 species/sample) from 14% to two (Figure 42a-b). Of the 17,955 pairwise combinations of pooled samples, 190 pairs (*i.e*. 1.1 %) had no species in common. By way of further example, shade plots of species abundance for pooled and un-pooled samples are provided in Figure 43a-b.

As for RB, species accumulation curves for EMB *a priori* areas suggested the current field design may be under-sampling (Figure 44). For all EMB areas, the accumulation curve for the total number of species observed (Sobs) continued to rise, and based on the predictions of several non-parametric (Chao1, 2, Jacknife 1, 2, Bootstrap) and parametric (MM) extrapolators, the probable number of species present in each area was likely to be considerably higher.

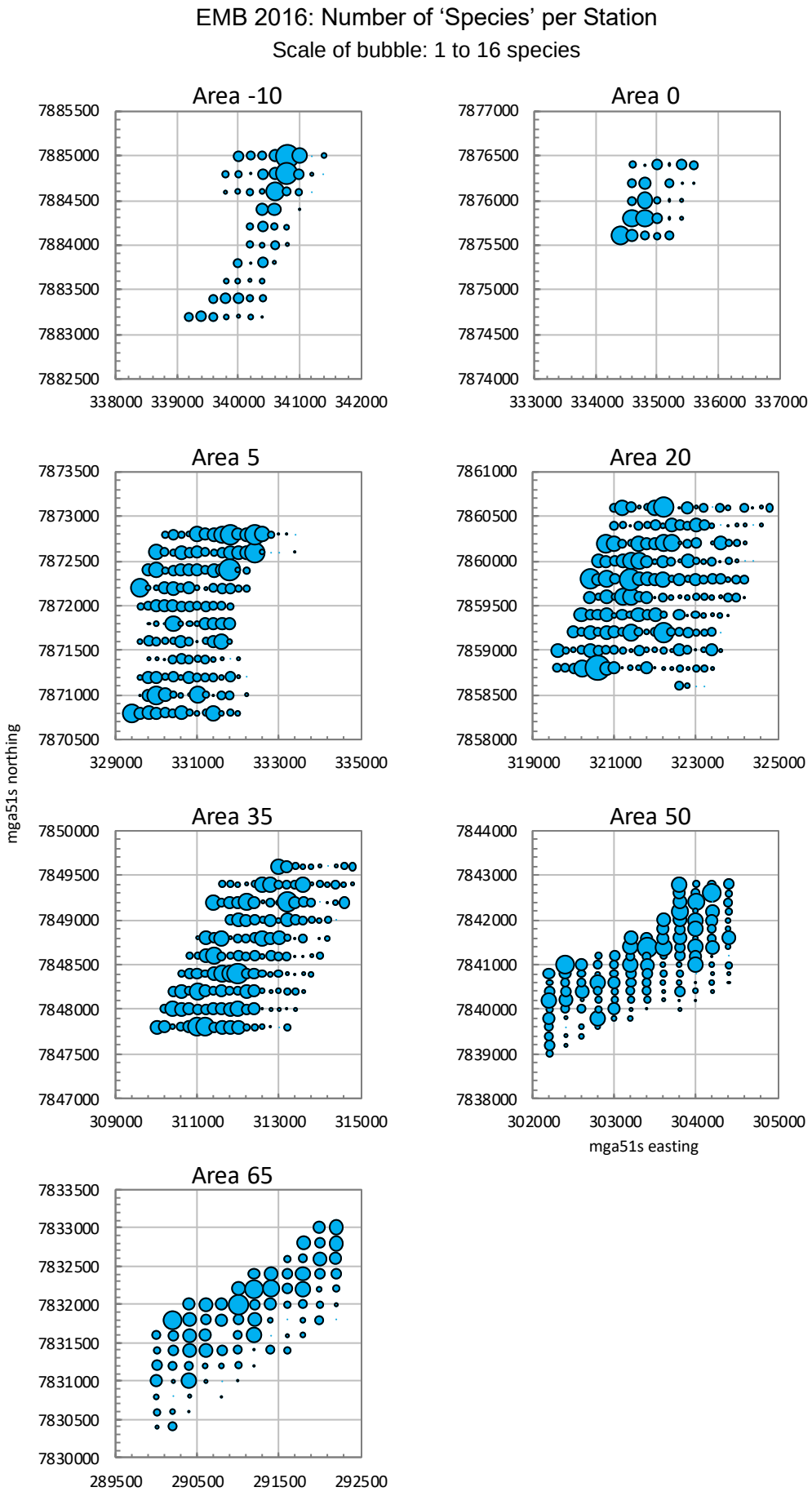


Figure 39a. EMB 2016: total number of benthic invertebrate taxa (species-level) recorded from each station in each area, based on un-pooled (raw) data.

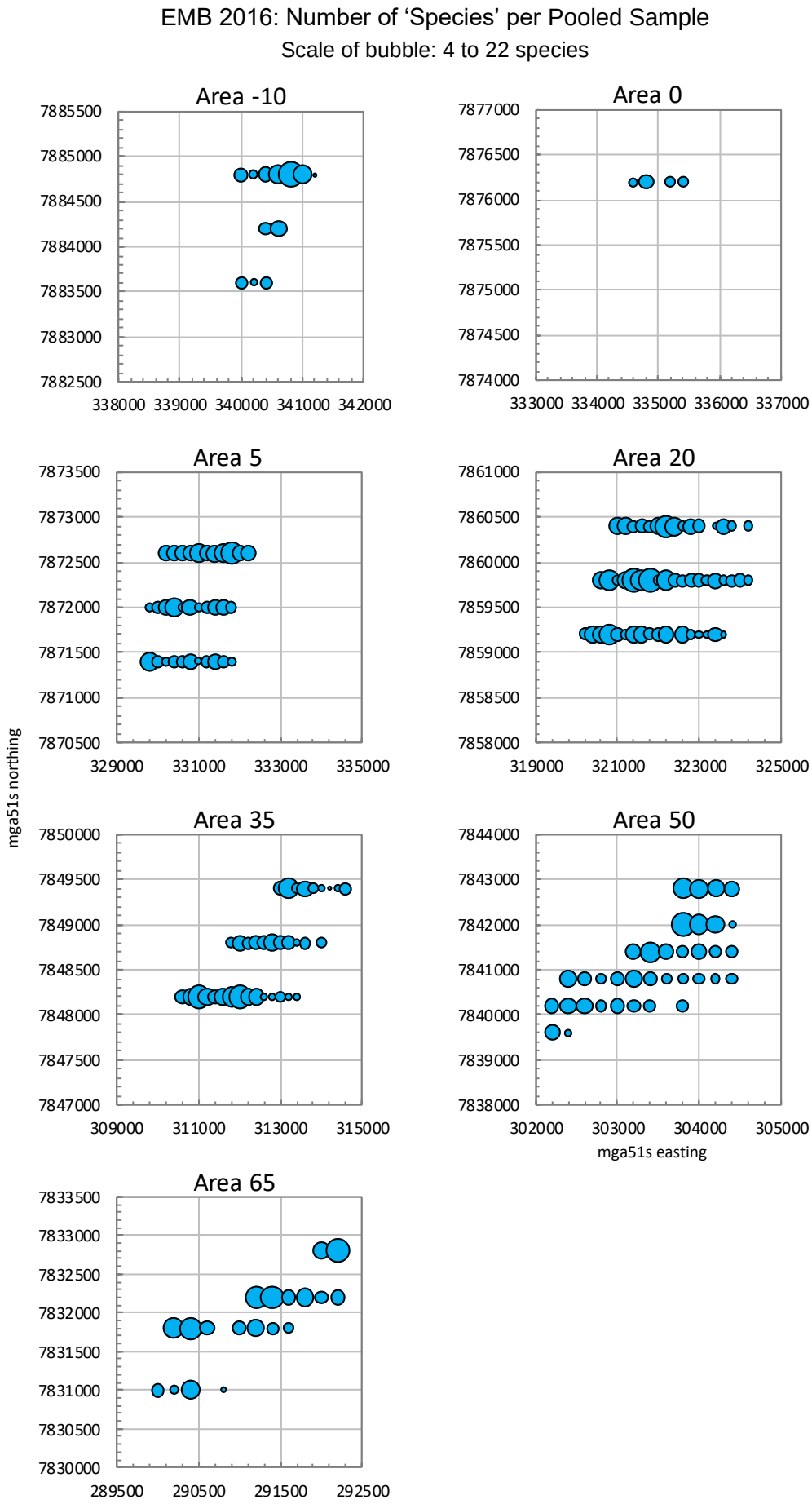


Figure 39b. EMB 2016: total number of benthic invertebrate taxa (species-level) calculated for each pooled sampled in each area.

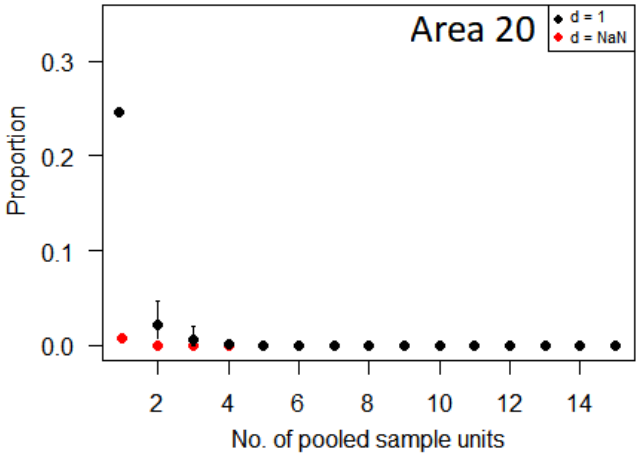


Figure 40. EMB 2016: proportion of Bray-Curtis dissimilarities equal to 1.0 (no species in common) or undefined (*i.e.*, NaN, "not a number") for area 20 with increasing numbers of samples being pooled together (from 1 to 14). Error bars indicate the 2.5 and 97.5 percentiles of the distribution of values obtained under 1000 permutations of the order of sampling units.

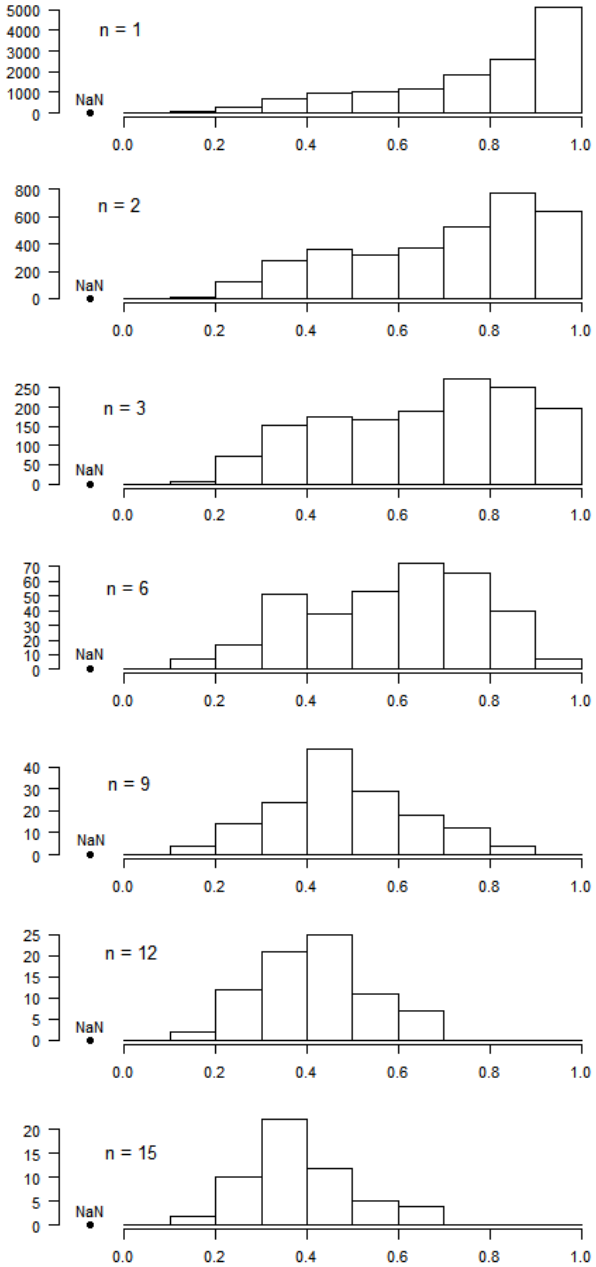
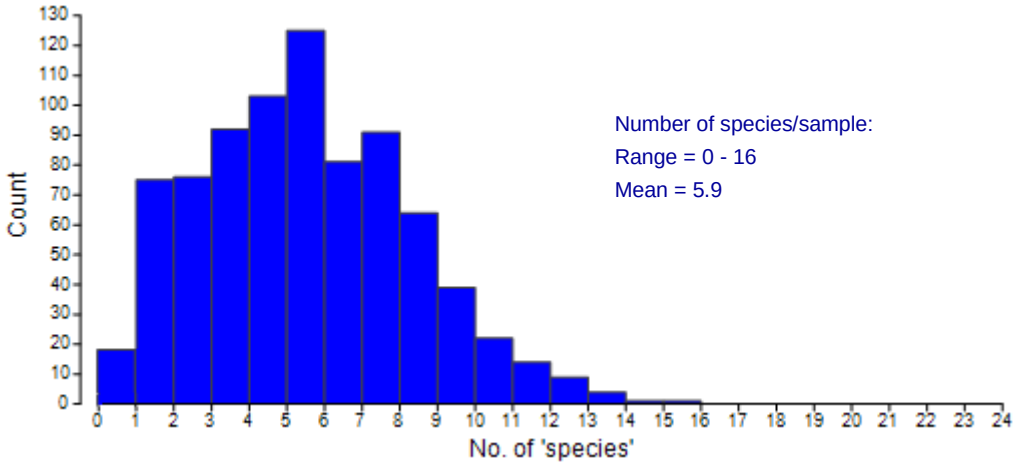


Figure 41. EMB 2016: Frequency distributions for Bray-Curtis dissimilarities for increasing numbers of samples from area 20 being pooled (*n* = 1 to 15). Pooling of samples was done merely in order of sample identification number.

(a) Sample size = 3 cores/sample



(b) Pooled samples = 9 cores/sample

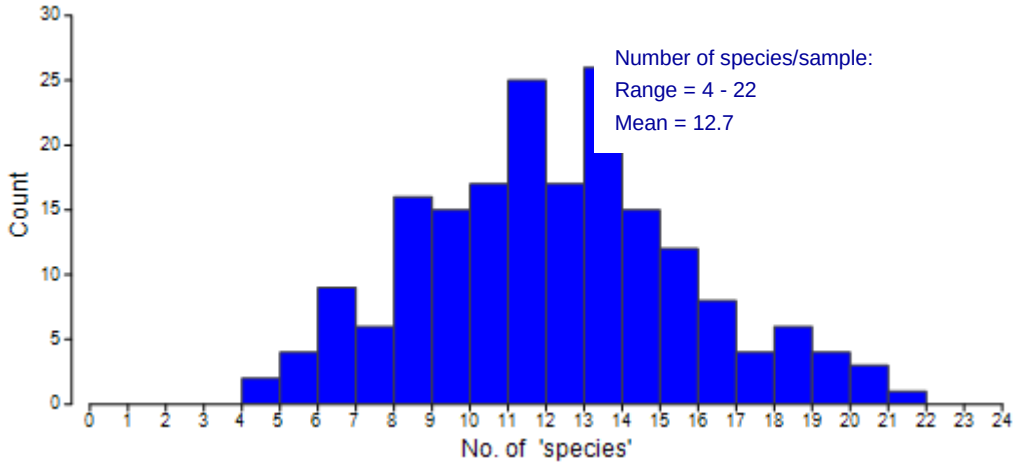


Figure 42. EMB 2016: frequency histograms for species richness (number of species) for (a) standard sample size of 3 cores, and (b) pooled sample size of 9 cores. Average and range in values for species richness across samples is also provided.

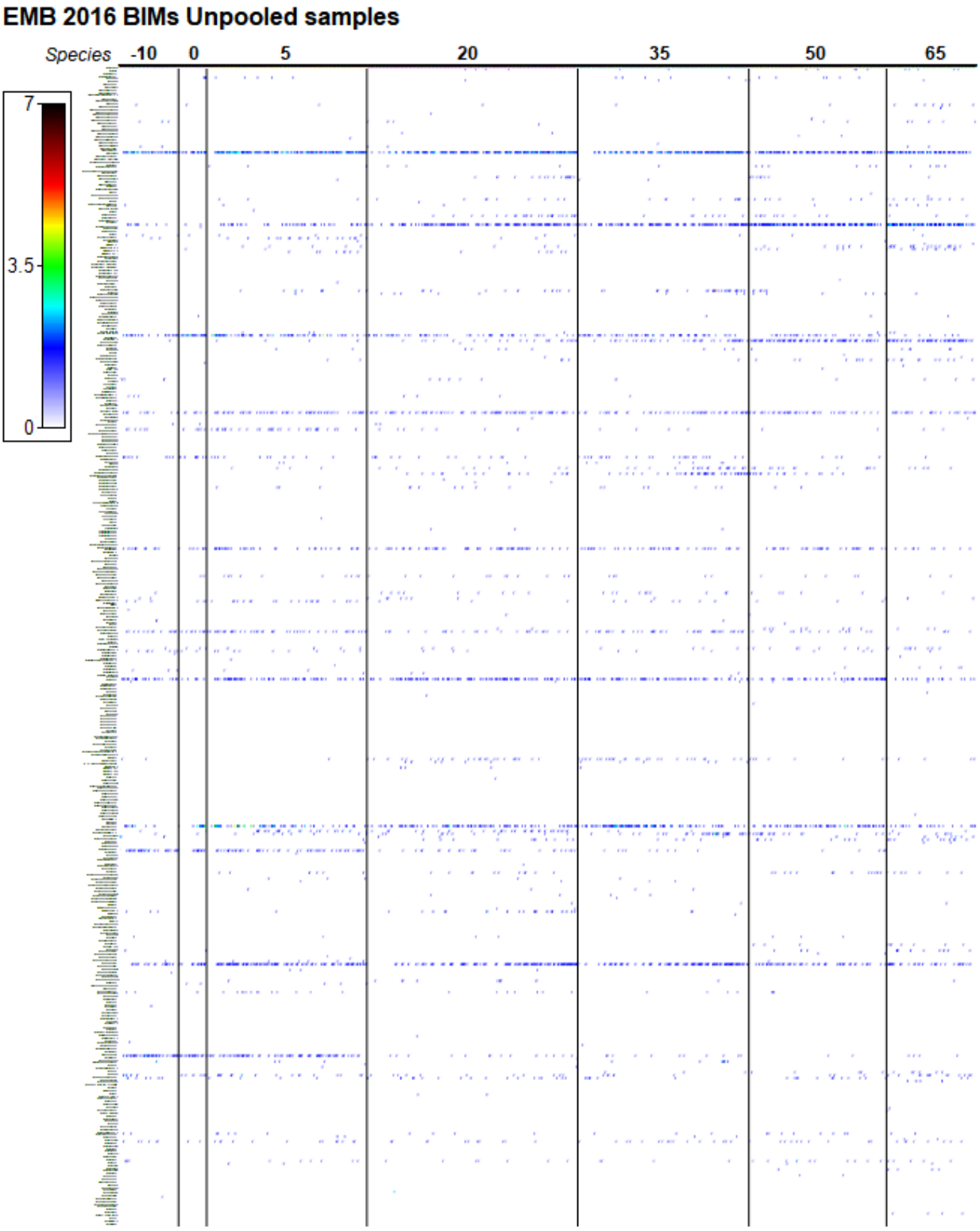


Figure 43a. EMB 2016: shade plot for species assemblage data for un-pooled samples, ordered by *a priori* area. Rectangle colours represent abundances (fourth-root transformed) on a continuously linear scale from absent (white) to the maximum value for the matrix (black). Note, species names are not intended to be legible, the gradation in 'shade' is the relevant attribute to interpret.

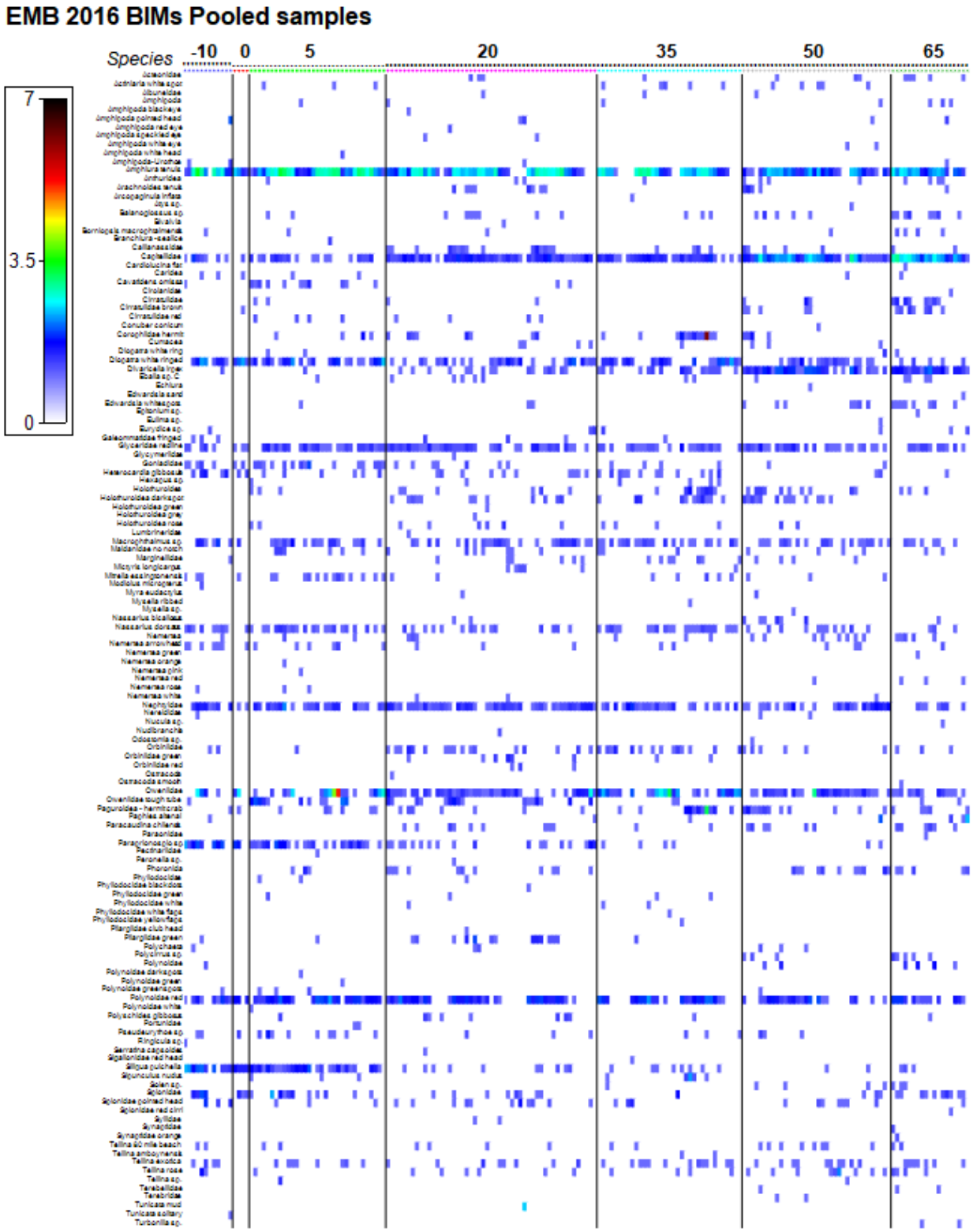


Figure 43b. EMB 2016: shade plot for species assemblage data for pooled samples, ordered by *a priori* area. Rectangle colours represent abundances (fourth-root transformed) on a continuously linear scale from absent (white) to the maximum value for the matrix (black). Note, species names are not intended to be legible, the gradation in 'shade' is the relevant attribute to interpret.

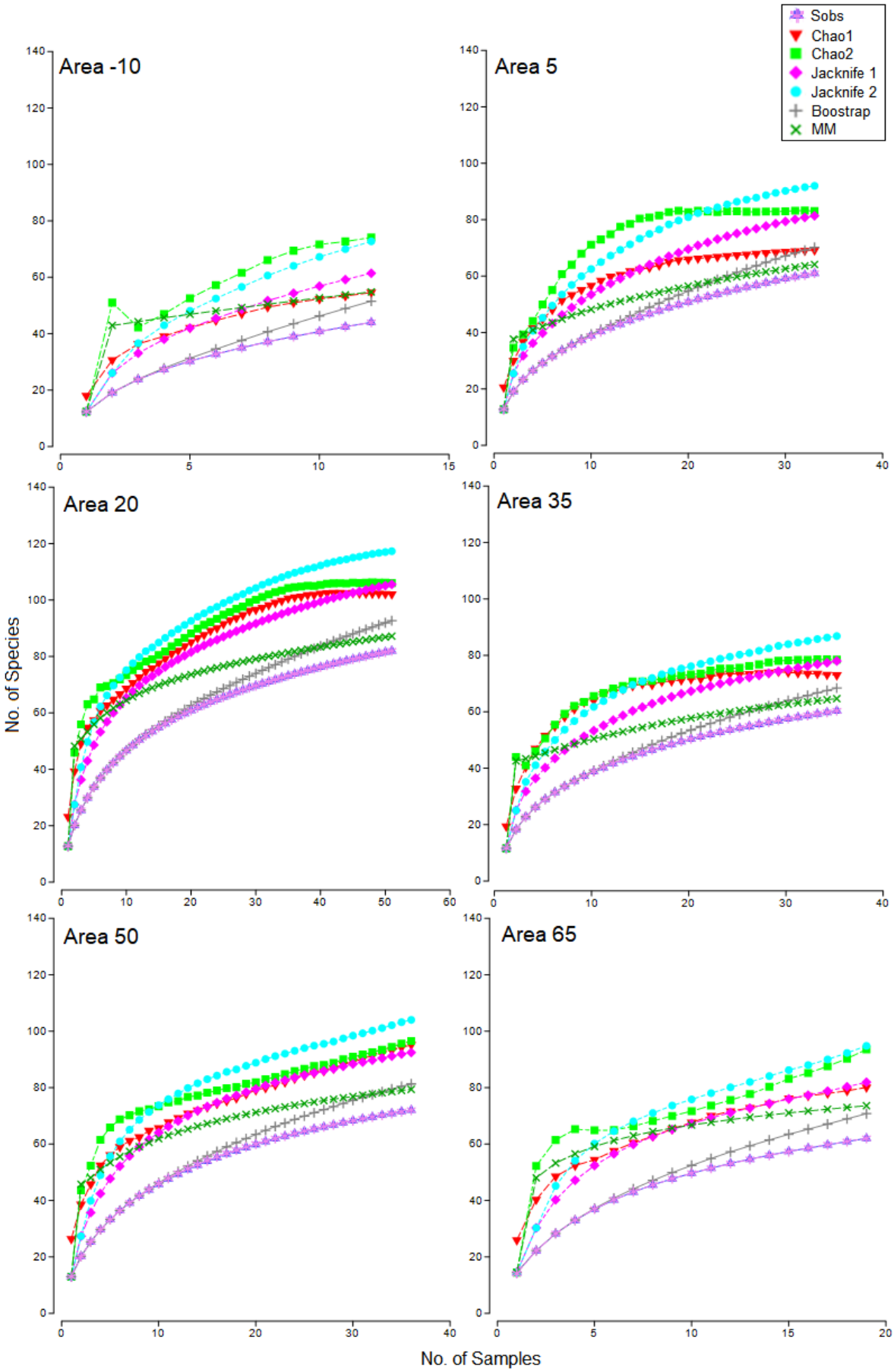


Figure 44. EMB 2016: species accumulation curves for each area based observed richness (Sob) in pooled samples and various richness estimators (Chao 1, Chao 2, Jacknife 1, Jacknife 2, Bootstrap, MM). Area 0 is not included as there were only a limited number of data points after samples were pooled.

4.3 Spatial Variability in Biota and Relationships with Environmental Factors

4.3.1 Univariate Metrics

Relationships with environmental variables were investigated using Spearman correlation (Table 11) and linear regression analyses on 2016 data (Table 12), and confirmed with scatter plots of species metrics on environmental data. Scatter plots for pooled as well as raw data are provided in (Appendix 2).

Spearman correlation using combined data for all areas, indicated there were moderate positive relationships with inundation for species richness (rho = 0.46, $p < 0.001$) and abundance (rho = 0.4, $p < 0.001$). Qualitative inundation category (0-30, 31-70, 71-100%) was also correlated with these species metrics, though the relationships were weaker (Table 11). The 70-100% inundation category was under-represented in the samples (refer Figure 33), so it was not unexpected that the categorical values were not as strongly correlated with species richness and abundance as continuous values. Stronger relationships with inundation (rho = 0.68 - 0.82, $p < 0.01$) were found within individual areas 20, 35, 50 and 65, with species richness and abundance generally increasing with increasing percent of time inundated up to ~60% inundation (Appendix 2). Above 60% inundation, species richness and abundance tended to decline. Scatter plots also showed bimodal distributions for evenness and, to a lesser degree, Shannon-Weiner diversity, across the range in inundation, with relatively lower values for evenness recorded in the mid-range for inundation (~40 to 60%) (Appendix 2).

Statistically significant relationships were also found for distance and penetrability (Table 11), however, these were weak (rho ≤ 0.26) with no clear consistent trends based on the scatter plots for either combined data for the beach or data for individual areas (Appendix 2).

Table 11. EMB 2016: significant results from Spearman correlation analyses for species diversity measures and environmental parameters, after pooling. Significant *p* values ($p < 0.05$) are highlighted blue for clarity.

Environmental parameter	No. of samples	Correlation	Species richness (*S*)	Total abundance (*N*)	Evenness (*J'*)	Shannon-Weiner (H' $\log_e$)
Inundation	190	Spearman rho	0.46	0.40	-0.27	0.01
		p value	<0.001	<0.001	<0.001	0.861
Inundation_category	190	Spearman rho	0.38	0.38	-0.30	-0.06
		p value	<0.001	<0.001	<0.001	0.452
Penetrability	190	Spearman rho	0.04	0.26	-0.19	-0.16
		p value	0.583	<0.001	0.008	0.029
Penetrability_category	190	Spearman rho	-0.05	0.21	-0.18	0.18
		p value	0.505	0.004	0.012	0.015

Stepwise multiple regression, using combined data for all areas, indicated inundation, distance and algae to have the best linear relationship with species richness, together accounting for 25.3% of total variance (Table 12). Inundation explained the most (12.0%) of the total variance in species evenness, while for species diversity, penetrability category was the only variable with a linear relationship, but explained only 2.9% of the total variance. There were no other significant linear relationships between species metrics and the measured environmental parameters (as expected based on the results of the Spearman correlations). The unique variance explained by continuous data for penetrability explained less than 5% of total variance in other species metrics (squared partial correlations; not shown).

Although the overall relationship between penetrability and species metrics was at best weak, it was still statistically significant and therefore penetrability, as well as inundation, was included as a covariable in ANCOVA testing for significant differences in species metrics among areas. Results indicated there were

significant area effects for species abundance but not for other species metrics, and there were no significant inundation or penetrability effects for any species metric (Table 13). In addition, the significant area effect for species abundance was not consistent when inundation and penetrability were taken into account (based on significant interactions terms for Area x Inund and Area x Penetr) (Table 13). Pairwise testing indicated the difference in species abundance among areas was solely due to the relatively small difference between area 5, where average abundance was highest (115.7 ± 24.4 SE), and areas 20 (58.6 ± 4.0 SE) and 50 (58.7 ± 6.2 SE), where average abundance was lowest. Plots in Figure 45a illustrate the differences in average values for species metrics among areas, together with 95% confidence intervals.

Table 12. EMB16: significant results from stepwise multiple regression of species data on environmental parameters. R = correlation value; %Var. = percentage of variance in species data explained.

Dependent	Independent	Model	ANOVA				R	%Var.
			df	MS	F	*p*		
Species Richness (*S*)	Inundation, Distance, Algae	Regression	3	202.92	21.00	<0.001	0.503	25.3
		Residual	186	9.662	--	--	--	--
		Total	189	--	--	--	--	--
Evenness (J')	Inundation, Inundation _cat	Regression	2	0.264	12.810	<0.001	0.347	12.0
		Residual	187	0.021	--	--	--	--
		Total	189	--	--	--	--	--
Diversity (*H'* $\log_e$)	Penetr_cat	Regression	1	0.943	5.674	0.018	0.171	2.9
		Residual	188	0.166				
		Total	189					

Table 13. ANCOVA testing for significant ($p < 0.05$) Area effects on EMB 2016 species richness, abundance ($\log_{10}$ transformed), diversity and evenness, after pooling, including inundation (Inund) and penetrability (Penetr) as covariables. Significant *p* values are highlighted blue for clarity.

Source	ANCOVA (main effects)				ANCOVA (main effects)			
	df	MS	F	*p*	df	MS	F	*p*
	Species richness				Species abundance			
Inundation	1	7.66	0.843	0.360	1	0.01	0.156	0.693
Penetrability	1	0.07	0.008	0.928	1	0.01	0.136	0.712
Area	6	8.33	0.917	0.484	6	0.18	3.061	0.007
Inund x Penetr	1	7.65	0.841	0.360	1	0.004	0.064	0.801
Area x Inund	6	7.80	0.858	0.527	6	0.15	2.498	0.024
Area x Penetr	6	5.49	0.604	0.727	6	0.14	2.423	0.029
Area x Inund x Penetr	6	6.87	0.756	0.606	6	0.11	1.856	0.092
Residual	162	9.09	--	--	162	0.06	--	--
Total	190	--	--	--	190	--	--	--
	Shannon-Weiner (*H'* $\log_e$)				Evenness (*J'*)			
Inundation	1	0.29	1.904	0.170	1	0.02	1.150	0.285
Penetrability	1	0.04	0.258	0.612	1	0.002	0.111	0.740
Area	6	0.15	0.976	0.443	6	0.03	1.598	0.151
Inund x Penetr	1	0.28	1.861	0.174	1	0.02	1.014	0.315
Area x Inund	6	0.10	0.672	0.672	6	0.02	0.802	0.569
Area x Penetr	6	0.07	0.458	0.839	6	0.02	0.988	0.436
Area x Inund x Penetr	6	0.10	0.626	0.709	6	0.01	0.502	0.806
Residual	162	0.15	--	--	162	0.02	--	--
Total	190	--	--	--	190	--	--	--

ANCOVA testing within areas showed there were no significant differences between sub-areas (Appendix 4). Plots of average values for species metrics for each sub-area are provided in Figure 45b. However, there were significant inundation effects within most areas (Appendix 4). Zones inundated for ≤ 30% of the time generally supported lower species richness and abundance than zones inundated for > 30 % of the time. The difference was most marked for abundance, with the lower inundation category zone (0 - 30%) typically supporting less than half the abundance of higher inundation zones (31 - 70%, 71 - 100%).

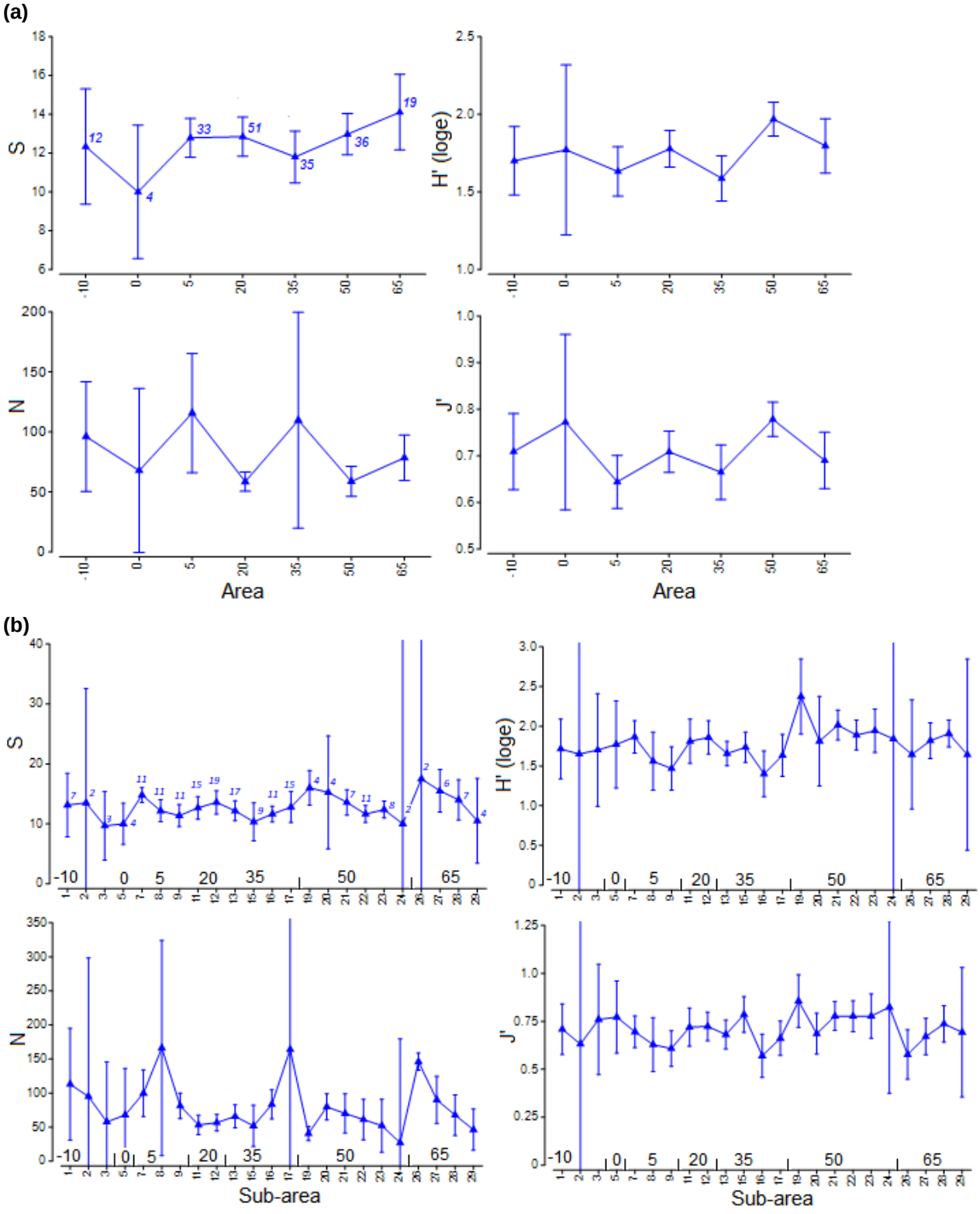


Figure 45. EMB16: Diversity indices (average ± 95% CI) for species-level data (after pooling) for, (a) each *a priori* area, and (b) each *a priori* sub-area. *S* = total number of species; *N* = total number of individuals; *H'* $\log_e$ = Shannon-Wiener diversity; *J'* = Pielou's evenness index. Number of samples after pooling is provided on each of the species richness plots.

Effect of algal cover was examined for those areas within which there were zones with and without algal cover, *i.e*. areas -10 and 20. Two-factor ANOVA testing (Area x Algae) showed no significant algal effect for any of the species metric ($p \geq 0.165$), and the non-significant interaction terms ($p \geq 0.095$) indicated this was consistent between the areas.

4.3.2 Multivariate Metrics

Exploratory analysis using k-R clustering together with the SIMPROF test suggested the EMB benthic invertebrate community could be broadly divided into five groups ($p < 0.05$) that appeared to loosely corresponded to geographic location and inundation zone, as depicted in Figures 46a-b.

Multivariate multiple regression analysis (DistLM) identified three environmental parameters with significant ($p = 0.001$) relationships with species assemblages: distance along the beach, inundation and penetrability. Individually, the parameter that explained the greatest amount of variation in the species data was distance along the beach (9.76%), followed by penetrability (8.60%) and inundation (8.27%); together accounting for 21.71% of the total variation (Table 14).

Table 14. Results from multivariate multiple regression (DistLM) of EMB 2016 species assemblage data on environmental parameters for (a) each parameter individually, and (b) stepwise selection of parameters. %Var. = percentage of variance in species data explained; Cum.(%) = cumulative percentage of variance explained.

Parameter	Pseudo F	*p*	%Var.	Cum. (%)
(a) Parameters individually				
Inundation	16.952	0.001	8.27%	
Penetrability	17.698	0.001	8.60%	
Distance (m)	20.321	0.001	9.76%	
(b) Parameters fitted sequentially				
Distance (m)	20.321	0.001	9.76%	
Inundation	16.952	0.001	8.69%	18.44%
Penetrability	17.698	0.001	3.27%	21.71%

Despite only explaining a very low percentage of the total variation, both penetrability and inundation appeared to have a significant influence on species assemblages within individual areas. In Figure 47, the dbRDA ordination plot for species abundance data versus penetrability and inundation shows samples separated by distance and penetrability along the x-axis (dbRDA1 axis) with samples from relatively higher penetrability areas (*i.e.* areas -10 and 0) toward the left side of the plot. Within areas samples separated by inundation along the y-axis (dbRDA2 axis) (Figure 47).

Species best correlated with the ordination axes were the razor clam *Siliqua pulchella* (Pearson r = 0.69) and the polychaete *Paraprionospio* sp. (r = 0.61), both of which were more common in higher penetrability-higher inundation zones (Figure 47) within the areas these species occurred. Capitellid polychaetes (r = 0.65) and the bivalve *Divaricella irpex* (r = 0.64) had higher abundance in area 65. Distributions of these species are shown in Figure 48. The long-armed brittle star *Amphiura tenuis* was also weakly correlated with higher penetrability-higher inundation zones (r = 0.50) (Figure 47). Again, caution must be used when interpreting the dbRDA plot, as the percentage of the variation explained by each of the axes is very low (≤ 10.5% of total variation). As for the RB analyses, the dbRDA plot for EMB is included to help visualise the relative importance of penetrability and inundation to the patterns of variation in the species assemblage data.

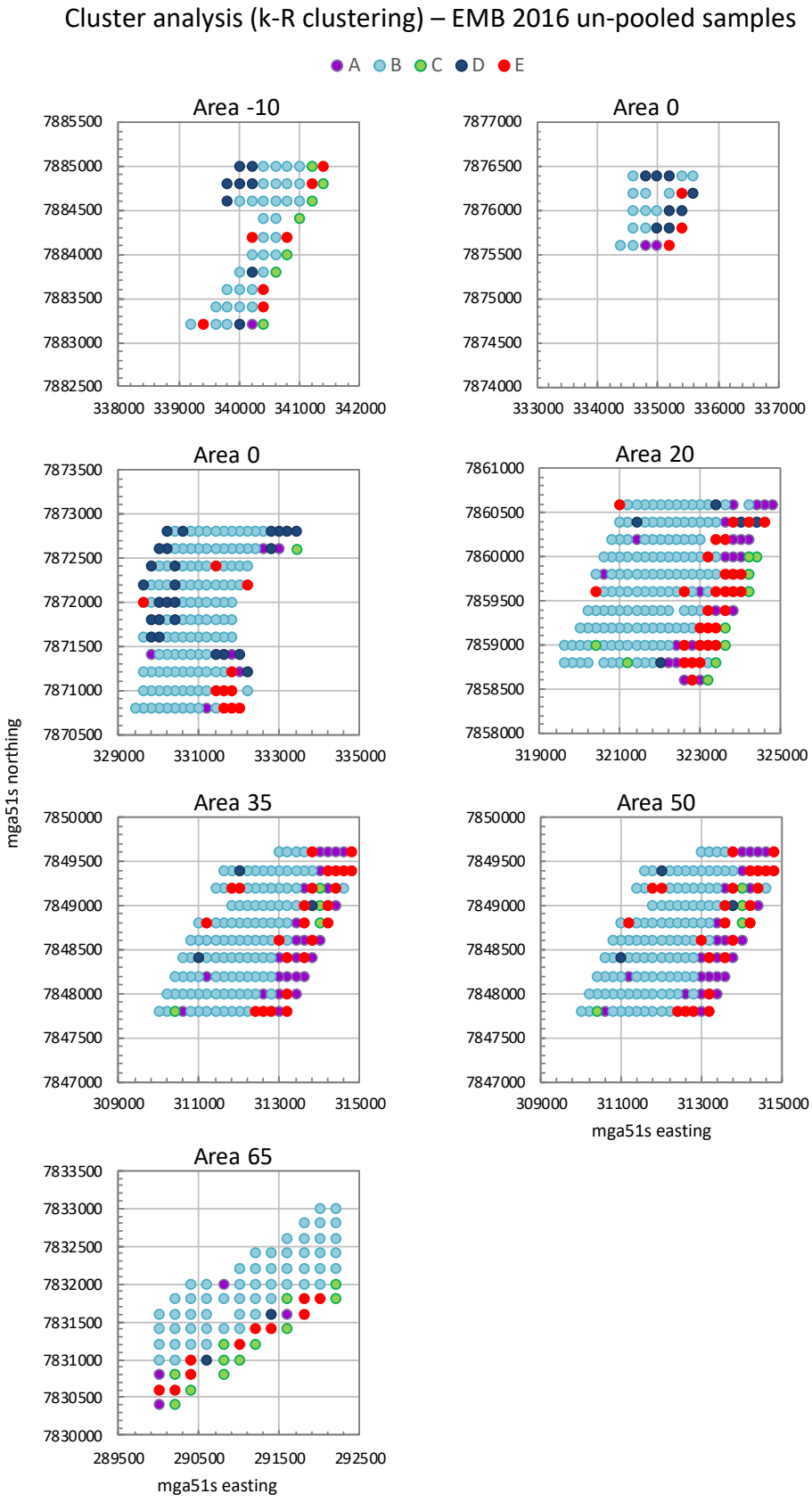


Figure 46a. EMB 2016: station groupings (A, B, C, D, E) determined from k-R clustering (non-hierarchical) on species assemblages (4th root transformed abundance), based on Bray-Curtis similarity for un-pooled samples.

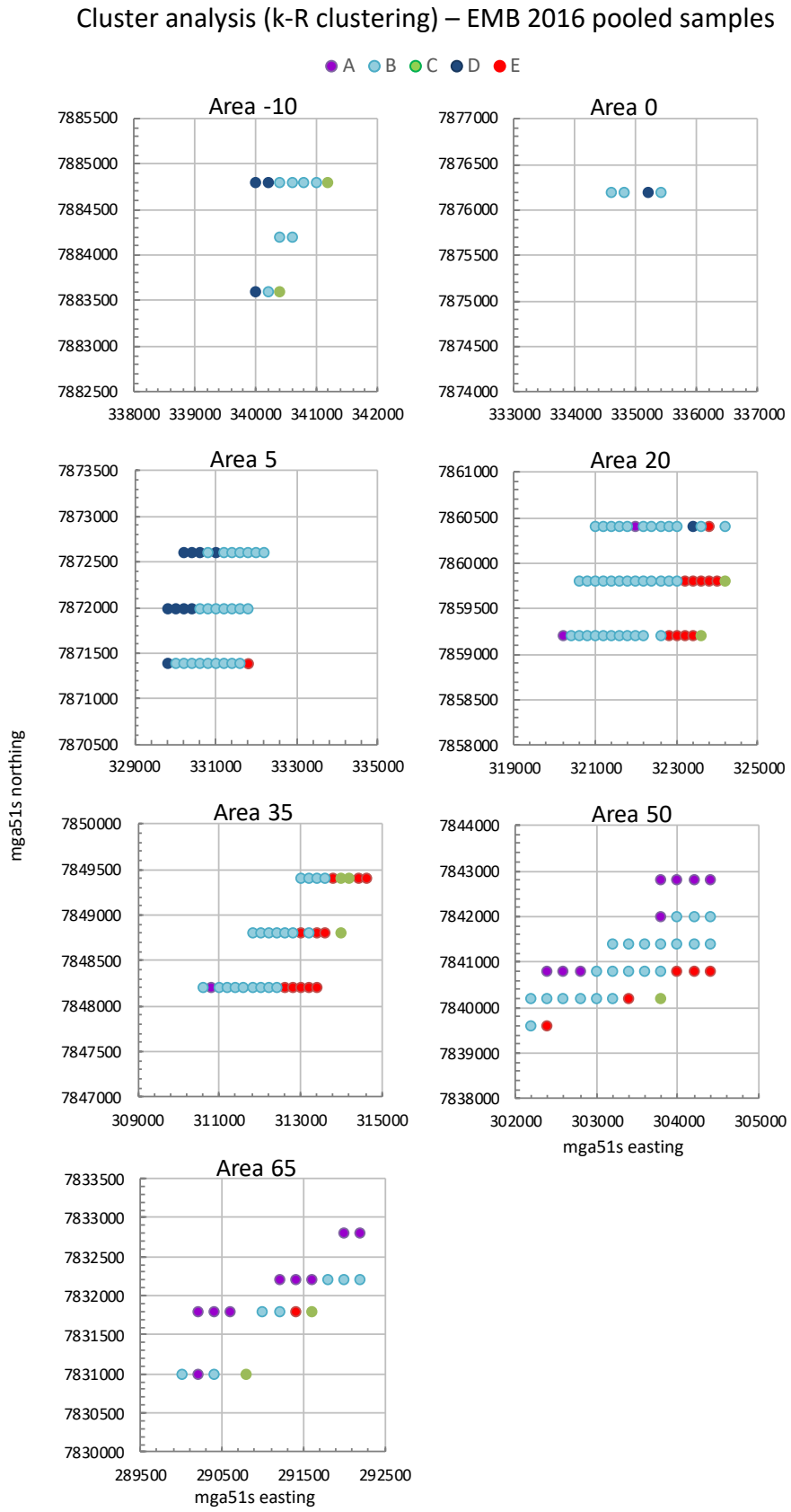


Figure 46b. EMB 2016: station groupings (A, B, C, D, E) determined from k-R clustering (non-hierarchical) on species assemblages (4th root transformed abundance), based on Bray-Curtis similarity for pooled samples.

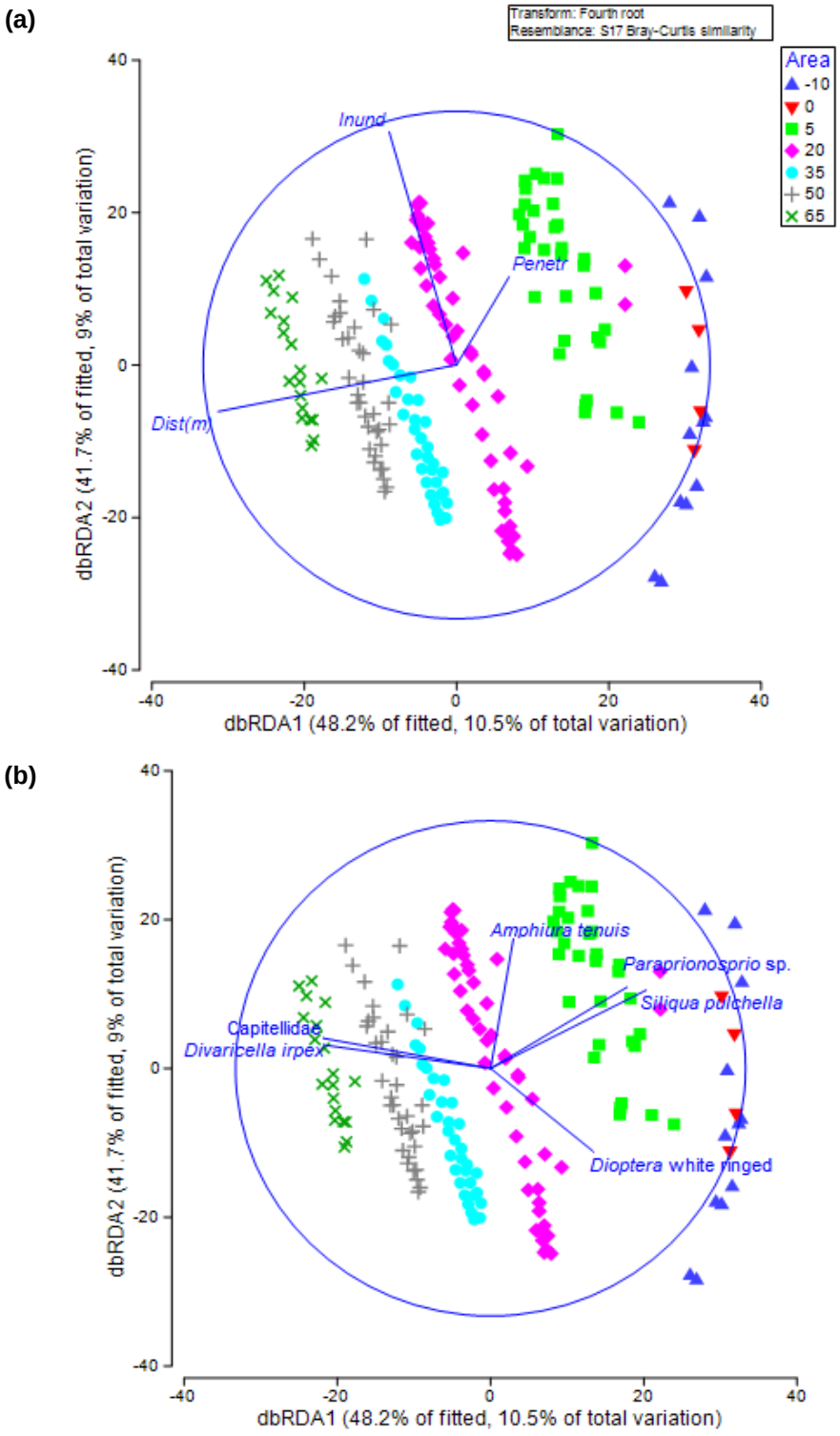


Figure 47. EMB 2016: dbRDA ordination for the fitted model on species abundance data versus penetrability (penetr) and inundation (inund), with vector overlays of (a) environmental variables (Pearson r > 0.89), and (b) individual taxa (Pearson coefficient: 0.50 < r < 0.70) best correlated with the ordination axes.

PERMANOVA testing indicated there were significant area effects for species assemblages, when inundation and penetrability were included as covariables (Table 15). Pairwise comparisons (not shown) indicated significant differences between most *a priori* areas ($p \leq 0.002$), except areas -10, 0 and 5 which were not significantly different to each other ($p \geq 0.063$), but were significantly different to all other areas.

Both penetrability and inundation were statistically significant and the strength of the effect was similar for both parameters, as indicated by the relative size of the estimated components of variation (*i.e.* penetrability 161.5, inundation 157.0; Table 15). While penetrability effects were consistent across areas (evidenced by the non-significant interaction term, Area x Penetr, $p = 0.663$), inundation effects were variable (Area x Inund, $p = 0.001$). Effects of penetrability were also variable for inundation (Inund x Penetr, $p = 0.001$) but the three-way interaction was not significant (Area x Inund x Penetr, $p = 0.126$).

Overall however, inundation and penetrability effects were less important components of the total variation among samples than were area effects (275), and all three were small components in comparison to the large amount of unexplained variation as indicated by the relatively large Residual (1386) (Table 15). Average pairwise percent similarity (Bray-Curtis) between areas ranged from 26.7%, between area -10 and 65, to 49.0%, between area 0 and 5 (Table 16). Contributing most to the difference between area -10 and 65 was the lower abundance of razor clam *S. pulchella,* but higher abundance of capitellid polychaetes and bivalve *Divaricella irpex* in area 65 (Figure 48). However, together these species contributed only 17% to the total variation between these areas, with respective contributions of 5.5%, 6.3% and 5.3% for each taxa. By comparison, taxa contributing most to the difference between area 0 and 5 were the polychaetes Oweniidae (7.5%), *Diopatra* sp. (white ringed) (5.9%), *Paraprionospio* sp. (5.8%) and Spionidae (5.1%), and the brittle star *A. tenuis* (5.0%), together accounting for 29% of the total variation. Average pairwise percent similarity within areas was only slightly higher than that between areas, ranging from 39.7% for area -10, to 51.7% for area 0 (Table 16).

Table 15. PERMANOVA testing for significant ($p < 0.05$) Area effects on EMB species assemblages (4^{th} root transformed abundance) including inundation (Inund) and penetrability (Penetr) categories as covariables.

Source	PERMANOVA main effects				Estimated Components of Variation	
	df	MS	Pseudo F	*p*	Var.	SD
Inundation	1	31221	22.531	0.001	157.0	12.5
Penetrability	1	30000	21.650	0.001	161.5	12.7
Area	6	7401	5.341	0.001	275.1	16.6
Inund x Penetr	1	7652	5.522	0.001	60.8	7.8
Area x Inund	6	3663	2.644	0.001	182.0	13.5
Area x Penetr	6	1281	0.9243	0.663	-35.8	-6.0
Area x Inund x Penetr	6	1674	1.208	0.126	219.9	14.8
Residual	162	1386	--	--	1385.7	37.2
Total	189	--	--	--	--	--

Table 16. EMB 2016: average pairwise percent similarity (Bray-Curtis) between/within areas.

	-10	0	5	20	35	50	65
-10	39.7						
0	45.4	51.7					
5	43.0	49.0	48.5				
20	34.1	36.5	39.6	42.3			
35	33.6	35.9	38.1	39.7	41.0		
50	29.8	31.6	34.3	39.7	39.1	43.7	
65	26.7	29.1	30.5	35.0	32.9	40.9	43.4

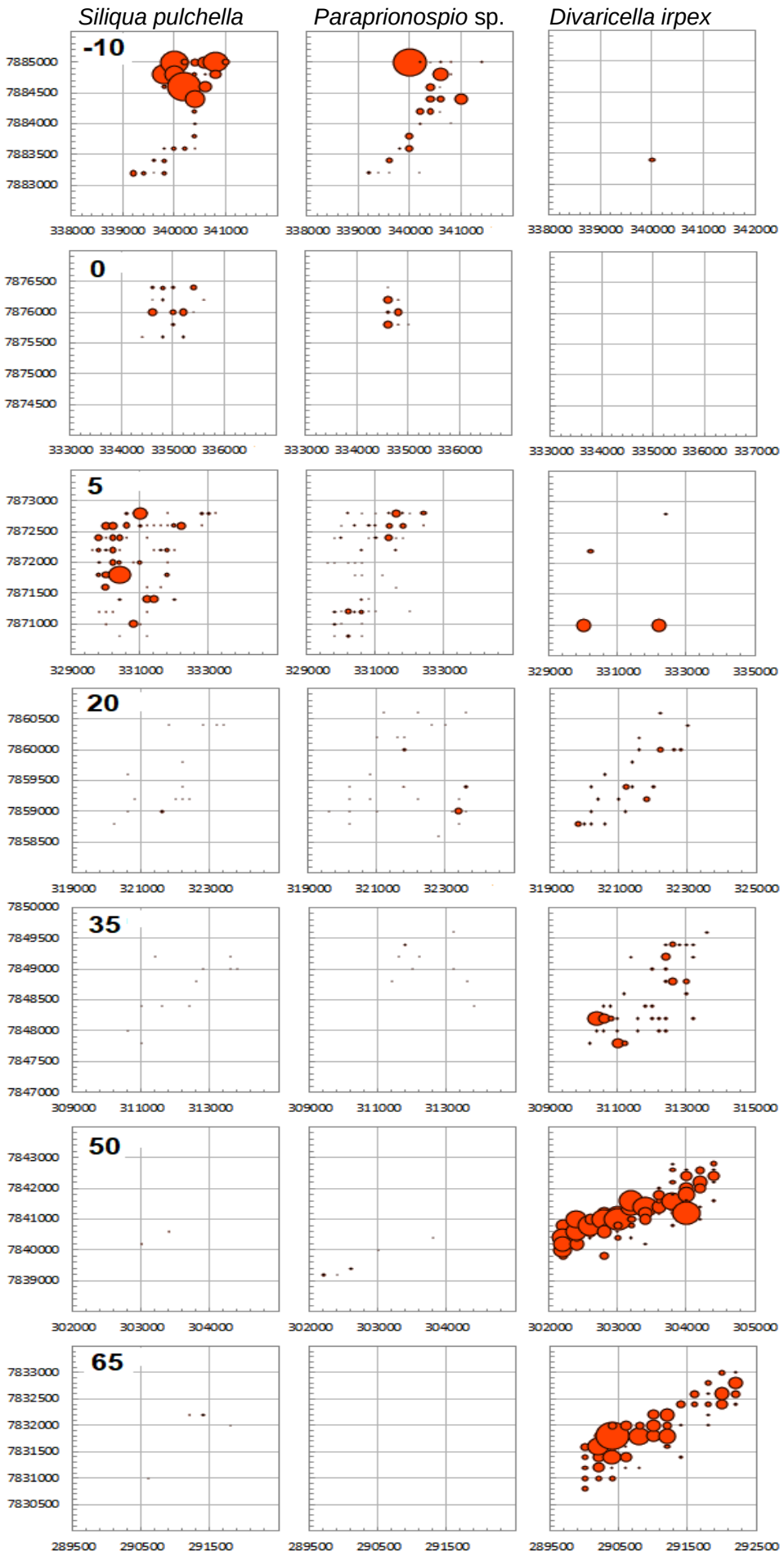


Figure 48. EMB 2016: distribution and abundance of the razor clam *Siliqua pulchella* (scale of bubble 1 to 29), the polychaete *Paraprionospio* sp. (scale of bubble 1 to 29) and the lucinid bivalve *Divaricella irpex* (scale of bubble 1 to 11).

The plot of the mMDS ordination of distances among centroids for areas, together with plots of the mMDS ordinations of distances among centroids for penetrability and inundation categories for areas are shown in Figure 49. The plots show the overlap in species assemblages among the inundation and penetrability categories. The same basic pattern is however, visible in all three plots, with areas -10, 0 and 5 tending to separate to the left along axis 1, and areas 20, 35, 50 and 65 to the right along axis 1. These stronger area effects were reflected in the separation of lower penetrability areas 35, 50, 65, and some parts of area 20, from higher penetrability areas 0, 5 and some parts of area -10. There was also some tendency for inundation categories to separate along axis 2, with samples from zones with shorter inundation periods (0-30%) toward the bottom.

PERMANOVA testing also indicated there were significant differences among sub-areas within areas (Appendix 5), and pairwise tests and interaction terms suggested these differences were related to penetrability effects within area -10, and inundation effects within areas 5 to 65. The plot of mMDS ordination of distances among centroids for sub-areas (Figure 50) illustrates the patterns indicated by the PERMANOVA results. Penetrability appeared to be a significant factor influencing samples from the northern end of area -10 (sub-area 1) in comparison to those from the southern end of area -10 (sub-area 3). Penetrability effects were not significant within other areas. Inundation similarly appeared to be a significant factor in the difference between some sub-areas within areas 20 (*i.e.* sub-area 11 and 13) and 50 (*i.e.* sub-areas, 19, 20 and 22). Inundation effects were also apparent within areas 5, 35 and 65 however, there were no statistically significant differences between sub-areas within each of these areas (Appendix 5). These results must be viewed with caution given the small sample size (n = 2 - 3) for some sub-areas (*i.e.* sub-area 2, 3, 24, 26).

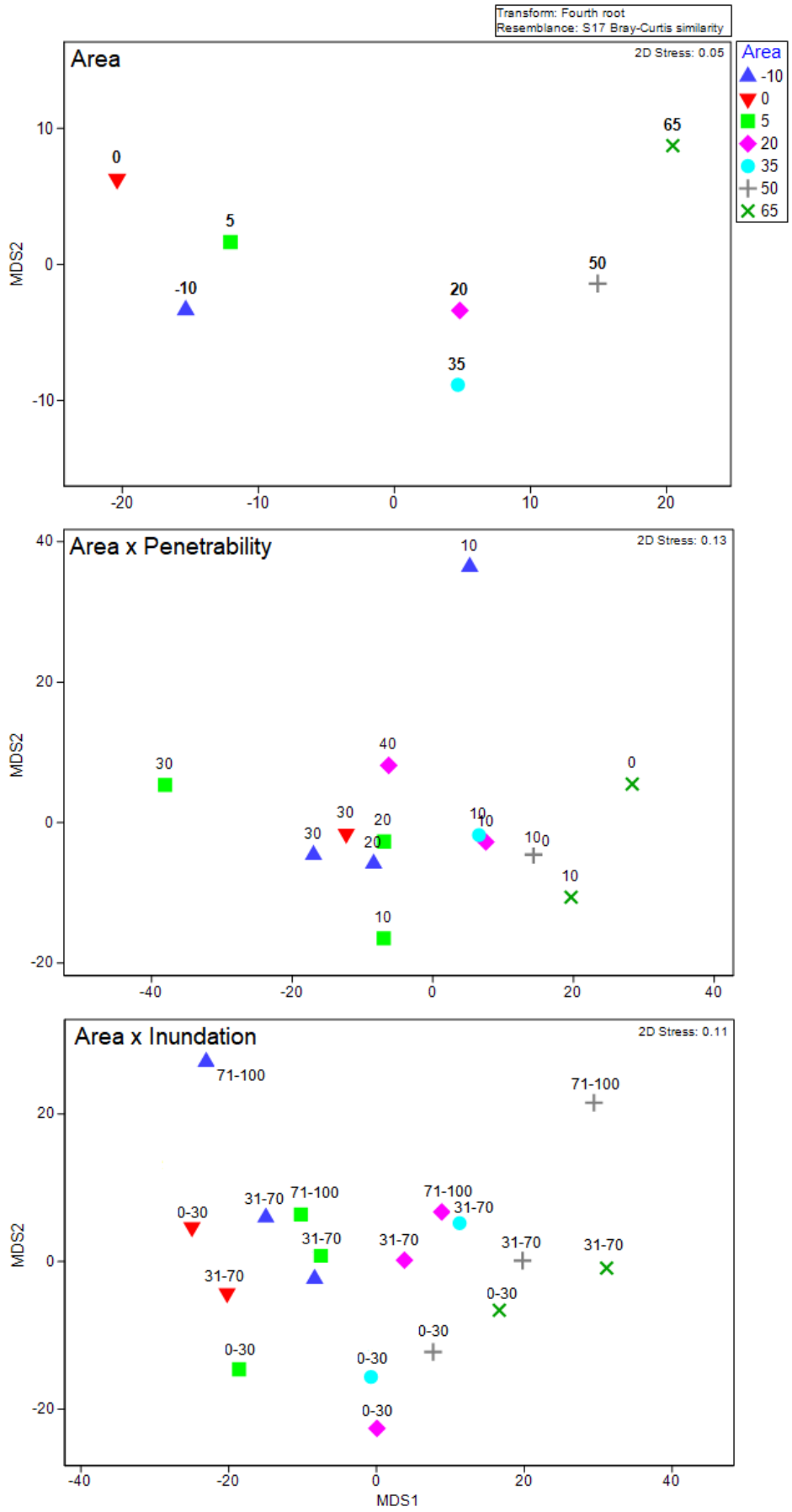


Figure 49. EMB 2016: mMDS ordination of distances among centroids for areas (top) penetrability categories (0, 10, 20, 20, 40 cm) for areas (middle) and inundation categories (30 = 0-30%, 70 = 31-70%, 100 = 71-100%) for areas (bottom), based on Bray-Curtis similarity (4th root transformed abundance). Samples are colour-coded by area and labelled by penetrability or inundation category. Optimum solution in 3D for Area with stress = 0.03, Area x Penetrability with stress = 0.07, and Area x Inundation with stress = 0.07.

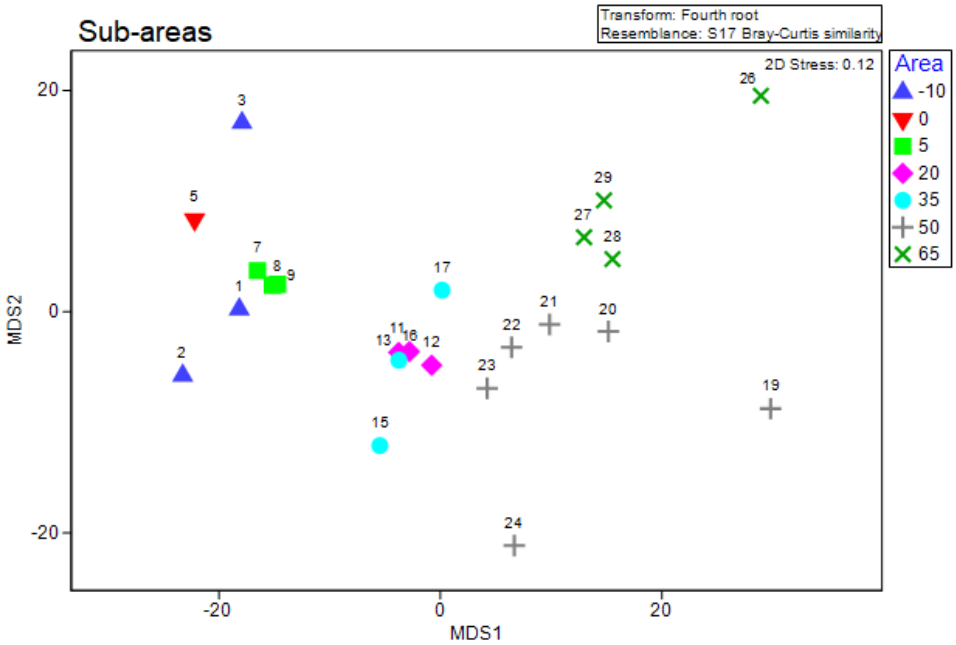


Figure 50. EMB 2016: mMDS ordination of distances among centroids for *a priori* sub-areas within areas, based on Bray-Curtis similarity (4th root transformed abundance). Samples are colour-coded by area and labelled by sub-area. Optimum solution in 3D with stress = 0.07.

4.4 Temporal Variability in Biota and Relationships with Environmental Factors

4.4.1 Univariate Metrics

Between-year changes (1999 versus 2016) in average (± 95% CI) values for species metrics for each area are shown in in Figure 51. Between 1999 and 2016, significant increases were recorded for species richness and abundance in area -10, 0, and 5, in species richness in area 20, 35 and 50, and in diversity in area 35 and 50 (two-factor ANOVA, Year x Sub-area, Table 17). Again, results for area 0 must be viewed with caution given the small number of samples for each of 1999 (3) and 2016 (4) after pooling.

The significantly higher species richness and abundance recorded in 2016, compared to 1999, was associated with a period of reduced frequency and intensity of tropical cyclones to cross the Kimberley between 2001 and 2016. The lower richness and abundance in 1999 may also reflect effects of Cyclone Vance in March 1999, which resulted in the loss of part of the beach near Anna Plains (Piersma *et al.* 2005). Anecdotal evidence also suggests the sediment particle size in other areas was altered due to the cyclone, with fine sediments washed away, but by 2016 there were more areas of deeper fine sediment than in 1999.

Significant sub-area effects were also detected for species richness within area 5 and area 50, which were consistent between years. In area 5 species richness was slightly but significantly higher in sub-area 7 (ave. 12.9 ± 0.79 SE) toward the northern end, compared to sub-areas 8 (ave. 10.6 ± 0.92 SE) and 9 (ave. 10.1 ± 0.95 SE). In area 50, the difference appeared related to a north-south gradient in species richness from sub-area 19 (ave. 15.6 ± 0.81 SE) to sub-area 24 (ave. 8.5 ± 1.89 SE).

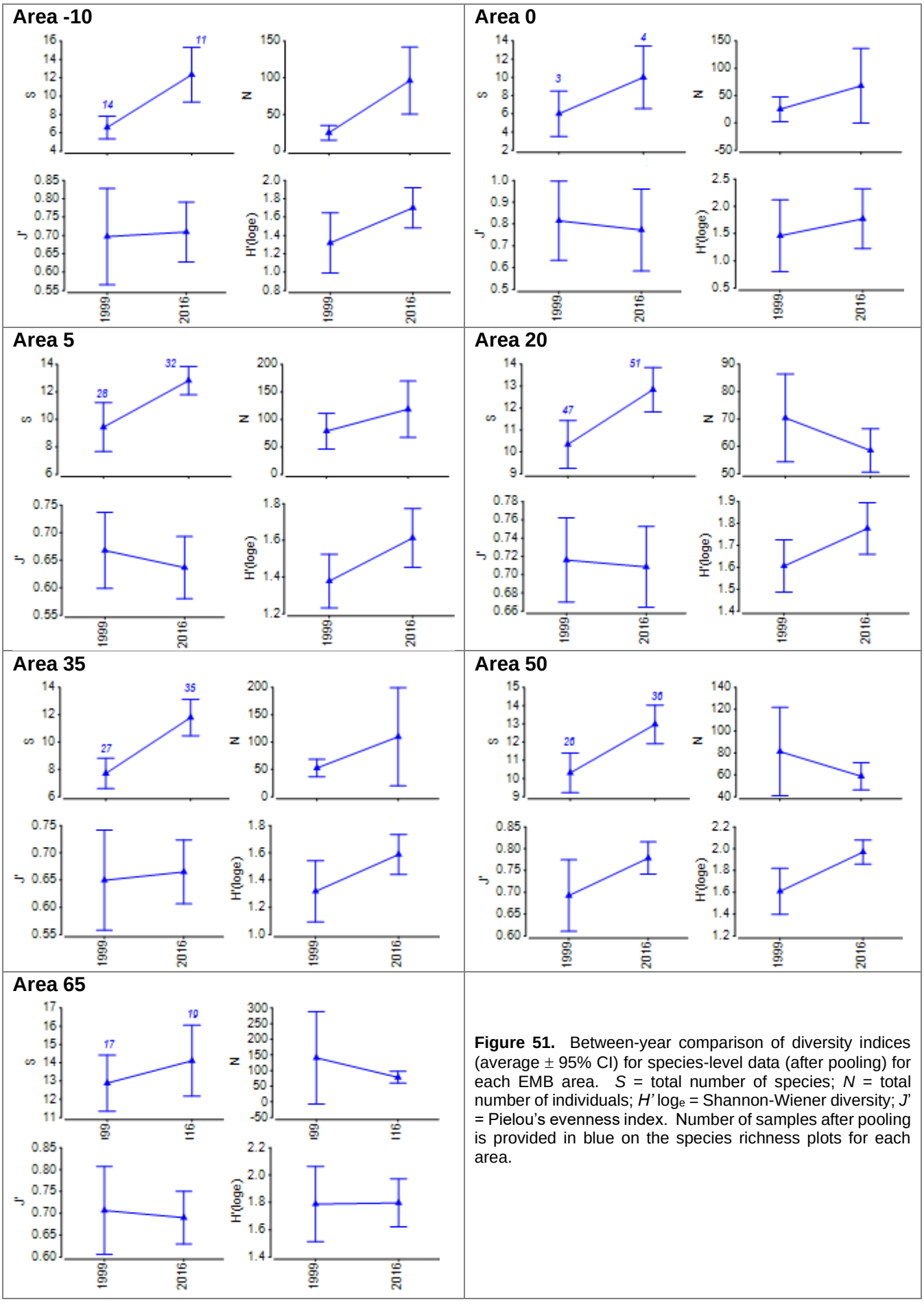


Figure 51. Between-year comparison of diversity indices (average ± 95% CI) for species-level data (after pooling) for each EMB area. *S* = total number of species; *N* = total number of individuals; *H'* $\log_e$ = Shannon-Wiener diversity; *J'* = Pielou's evenness index. Number of samples after pooling is provided in blue on the species richness plots for each area.

Table 17. Two-factor ANOVA testing for significant ($p < 0.05$) Year and Sub-area effects on species-level diversity indices (after pooling) for each EMB area (-10, 0, 5, 20, 35, 50, 65). *S* = species richness; *N* = total abundance (log_{10} transformed); *J'* = evenness (log_{10} transformed), H' (log_e) = Shannon-Weiner diversity. Significant *p* values are shaded blue for clarity.

Area	Source	Variable	ANOVA (main effects)			
			df	MS	F	*p*
-10	Year	*S*	1	173.285	12.937	0.002
		N	1	1.335	9.363	0.006
		J'	1	0.000	0.186	0.671
		H' (log$_e$)	1	0.990	3.985	0.060
	Sub-Area	*S*	2	5.242	0.391	0.681
		N	2	0.142	0.995	0.387
		J'	2	0.002	0.958	0.401
		H' (log$_e$)	2	0.139	0.559	0.580
	Year x Sub-Area	*S*	2	13.507	1.008	0.383
		N	2	0.012	0.085	0.919
		J'	2	0.001	0.527	0.598
		H' (log$_e$)	2	0.164	0.661	0.527
	Residual	*S*	20	13.395	--	--
		N	20	0.143	--	--
		J'	20	0.002	--	--
		H' (log$_e$)	20	0.248	--	--
	Total df (each metric) = 26					
0	Year	*S*	1	173.285	12.937	0.002
		N	1	1.335	9.363	0.006
		J'	1	0.000	0.186	0.671
		H' (log$_e$)	1	0.990	3.985	0.060
	Sub-Area	*S*	2	5.242	0.391	0.681
		N	2	0.142	0.995	0.387
		J'	2	0.002	0.958	0.401
		H' (log$_e$)	2	0.139	0.559	0.580
	Year x Sub-Area	*S*	2	13.507	1.008	0.383
		N	2	0.012	0.085	0.919
		J'	2	0.001	0.527	0.598
		H' (log$_e$)	2	0.164	0.661	0.527
	Residual	*S*	20	13.395	--	--
		N	20	0.143	--	--
		J'	20	0.002	--	--
		H' (log$_e$)	20	0.248	--	--
	Total df (each metric) = 26					
5	Year	*S*	1	173.367	12.978	0.001
		N	1	1.158	8.275	0.006
		J'	1	0.001	0.745	0.392
		H' (log$_e$)	1	0.739	4.783	0.033
	Sub-Area	*S*	2	43.886	3.285	0.045
		N	2	0.234	1.672	0.197
		J'	2	0.001	0.698	0.502
		H' (log$_e$)	2	0.022	0.145	0.865
	Year x Sub-Area	*S*	2	3.250	0.243	0.785
		N	2	0.332	2.376	0.103
		J'	2	0.010	5.611	0.006
		H' (log$_e$)	2	0.762	4.934	0.011
	Residual	*S*	54	13.358	--	--
		N	54	0.140	--	--
		J'	54	0.002	--	--
		H' (log$_e$)	54	0.154	--	--
	Total df (each metric) = 60					

Area	Source	Variable	ANOVA (main effects)			
			df	MS	F	*p*
20	Year	*S*	1	143.759	10.546	0.002
		N	1	0.001	0.011	0.917
		J'	1	0.000	0.049	0.826
		H' (log$_e$)	1	0.705	4.112	0.045
	Sub-Area	*S*	2	13.215	0.969	0.383
		N	2	0.135	1.512	0.226
		J'	2	0.000	0.027	0.974
		H' (log$_e$)	2	0.037	0.218	0.805
	Year x Sub-Area	*S*	2	2.042	0.150	0.861
		N	2	0.242	2.706	0.072
		J'	2	0.001	0.449	0.640
		H' (log$_e$)	2	0.188	1.099	0.338
	Residual	*S*	92	13.632	--	--
		N	92	0.089	--	--
		J'	92	0.002	--	--
		H' (log$_e$)	92	0.171	--	--
	Total df (each metric) = 98					
35	Year	*S*	1	143.759	10.546	0.002
		N	1	0.001	0.011	0.917
		J'	1	0.000	0.049	0.826
		H' (log$_e$)	1	0.705	4.112	0.045
	Sub-Area	*S*	2	13.215	0.969	0.383
		N	2	0.135	1.512	0.226
		J'	2	0.000	0.027	0.974
		H' (log$_e$)	2	0.037	0.218	0.805
	Year x Sub-Area	*S*	2	2.042	0.150	0.861
		N	2	0.242	2.706	0.072
		J'	2	0.001	0.449	0.640
		H' (log$_e$)	2	0.188	1.099	0.338
	Residual	*S*	92	13.632	--	--
		N	92	0.089	--	--
		J'	92	0.002	--	--
		H' (log$_e$)	92	0.171	--	--
	Total df (each metric) = 98					
50	Year	*S*	1	48.935	7.173	0.010
		N	1	0.094	0.893	0.349
		J'	1	0.007	3.543	0.066
		H' (log$_e$)	1	1.124	6.871	0.012
	Sub-Area	*S*	5	31.559	4.626	0.002
		N	5	0.065	0.620	0.685
		J'	5	0.001	0.342	0.885
		H' (log$_e$)	5	0.244	1.489	0.210
	Year x Sub-Area	*S*	5	1.524	0.223	0.951
		N	5	0.047	0.449	0.812
		J'	5	0.002	0.873	0.506
		H' (log$_e$)	5	0.162	0.992	0.432
	Residual	*S*	50	6.822	--	--
		N	50	0.105	--	--
		J'	50	0.002	--	--
		H' (log$_e$)	50	0.164	--	--
	Total df (each metric) = 62					
60	Year	*S*	1	22.516	1.874	0.182
		N	1	0.000	0.002	0.962
		J'	1	0.001	0.502	0.485
		H' (log$_e$)	1	0.021	0.103	0.750
	Sub-Area	*S*	3	10.882	0.906	0.451
		N	3	0.109	1.321	0.287
		J'	3	0.002	1.061	0.381

Area	Source	Variable	ANOVA (main effects)			
			df	MS	F	*p*
		H' (log$_e$)	3	0.092	0.460	0.713
	Year x Sub-Area	*S*	3	23.129	1.925	0.149
		N	3	0.204	2.474	0.082
		J'	3	0.005	2.867	0.054
		H' (log$_e$)	3	0.385	1.915	0.150
	Residual	*S*	28	12.018	--	--
		N	28	0.083	--	--
		J'	28	0.002	--	--
		H' (log$_e$)	28	0.201	--	--
	Total df (each metric) = 36					

4.4.2 Multivariate Metrics

There were significant between-year differences in species assemblages for all areas (PERMANOVA, Table 18). Within area 50, there was also significant differences in species assemblages among sub-areas, predominantly due to a north-south gradient in species assemblages, which was most well defined in 1999, though still apparent in 2016 (Figure 52). Similarity in faunal assemblages between 1999 and 2016 ranged from 20.1% for area 0, to 40.5% for area 65 (Table 19). The lower between-year similarity recorded for area 0 may have been influenced by the low number of samples for this area after pooling, *i.e*. 3 in 1999 and 4 in 2016.

The estimated components of variation (Table 18) suggested only about one third to one half of the total variation was attributable to specific year effects (as can be seen by the comparatively large Residuals). This was also the case for RB (section 3.4.2 Table 8). The most consistent temporal change among EMB areas was the increased abundance of *Diopatra* sp. (white ringed), *Paraprionospio* sp., and *Amphiura tenuis* in 2016 compared to 1999, though individually, these species each contributed less than 10% to the total variability within any given area.

Table 18. Two-factor PERMANOVA testing for significant ($p < 0.05$) Year and Sub-Area effects on EMB species assemblages (4th root transformed abundance) within each area (-10, 0, 5, 20, 35, 50, 65). Significant *p* values are shaded blue for clarity. Note there were insufficient number of samples (after pooling) to tests between sub-areas within areas 0.

Area	Source	PERMANOVA (main effects)				Estimated Components of Variation	
		df	MS	Pseudo-F	*p*	Var.	SD
-10	Year	1	7816	3.457	0.001	517.3	22.7
	Sub-Area	2	1810	0.800	0.709	-62.0	-7.9
	Year x Sub-Area	2	2251	0.995	0.438	-2.9	-1.7
	Residual	20	2261	--	--	2261.0	47.6
	Total	25	--	--	--		
0	Year	1	8888	9.301	0.028	2313.6	48.1
	Sub-Area	0		No test			
	Year x Sub-Area	0		No test			
	Residual	5	956	--	--	955.6	30.9
	Total	6	--	--	--		
5	Year	1	16473	9.522	0.001	497.0	22.3
	Sub-Area	2	2100	1.214	0.243	18.6	4.3
	Year x Sub-Area	2	1380	0.798	0.707	-35.3	-5.9
	Residual	54	1730	--	--	1730.0	41.6
	Total	59	--	--	--		
20	Year	1	31110	17.612	0.001	604.6	24.6
	Sub-Area	2	2021	1.144	0.265	7.8	2.8

Area	Source	PERMANOVA (main effects)				Estimated Components of Variation	
		df	MS	Pseudo-F	*p*	Var.	SD
	Year x Sub-Area	2	2448	1.386	0.114	42.0	6.5
	Residual	92	1766	--	--	1766.4	42.0
	Total	97	--	--	--		
35	Year	1	25494	15.761	0.001	802.3	28.3
	Sub-Area	2	3168	1.959	0.019	77.4	8.8
	Year x Sub-Area	2	2220	1.373	0.140	60.2	7.8
	Residual	56	1618	--	--	1617.5	40.2
	Total	61	--	--	--		
50	Year	1	9159	6.113	0.001	403.6	20.1
	Sub-Area	5	3226	2.153	0.001	189.7	13.8
	Year x Sub-Area	5	2455	1.639	0.006	210.1	14.5
	Residual	50	1498	--	--	1498.2	38.7
	Total	61	--	--	--		
65	Year	1	8959	6.830	0.001	520.1	22.8
	Sub-Area	3	1437	1.095	0.326	14.6	3.8
	Year x Sub-Area	3	1526	1.164	0.218	50.4	7.1
	Residual	28	1312	--	--	1311.8	36.2
	Total	35	--	--	--		

Table 19. EMB: average pairwise percent similarity (Bray-Curtis) between/within years for each area.

Area		1999	2016
-10	**1999**	33.1	
	2016	26.4	39.7
0	**1999**	65.4	
	2016	20.1	51.7
5	**1999**	36.4	
	2016	34.3	49.4
20	**1999**	42.3	
	2016	32.3	42.3
35	**1999**	47.4	
	2016	30.5	41.0
50	**1999**	41.2	
	2016	33.3	43.7
65	**1999**	57.5	
	2016	40.5	43.4

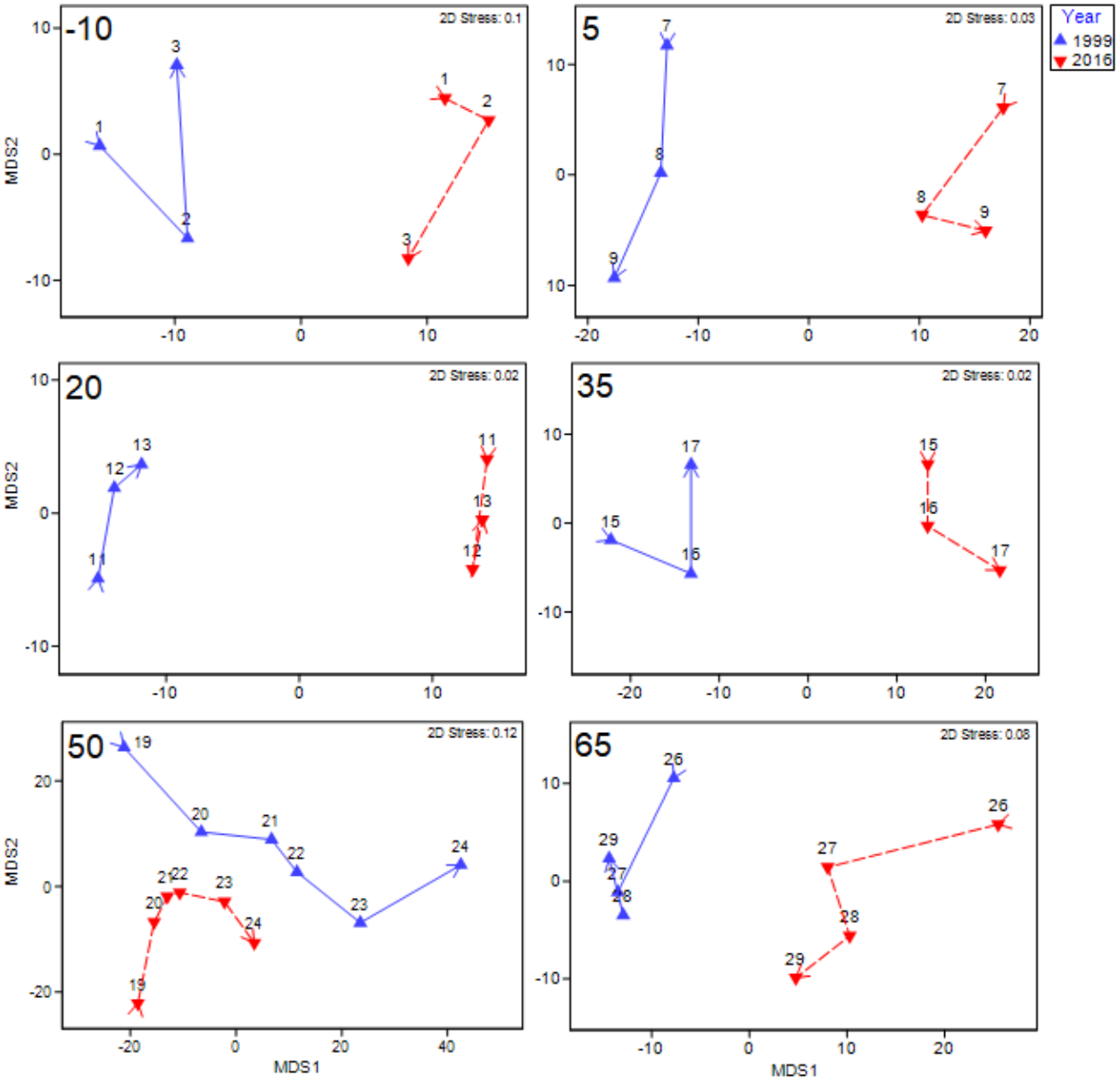


Figure 52. EMB: mMDS ordinations of distances among sub-area centroids for each *a priori* area, overlain with trajectory of change in species assemblages between sub-areas . Ordinations based on Bray-Curtis similarity (4th root transformed abundance). Samples are colour-coded by year and labelled by sub-area. Optimum solutions in 3D with stress for area -10 = 0.04, area 5 = 0.02, area 20 = 0.01, area 35 = 0.01, area 50 = 0.07, and area 65 = 0.05.

4.5 Detectable Change

Based on the same approach used for RB (section 3.5), it was assumed that power to detect temporal change at EMB would be similar to that calculated for MonRoeb16 data, if 9 cores per site were collected at EMB instead of the current 3 cores per site. To determine if detectable change would be less than temporal variability at EMB, percent change between years was calculated for the BIM data for 1999 and 2016 (Figure 53). Note that there are no monthly MonRoeb data for EMB than could be used to investigate effects of changes in sample size on power, and it wasn't deemed appropriate to use the AnnRoeBIM data without spatially confounding the data due to the pooling of adjacent sites. Average values for univariate species metrics within each EMB area (-10, 0, 5, 20, 35, 50, 65), typically varied by more than 20% each year and was particularly large for species richness and abundance, relative to evenness and diversity measures. Therefore, a sampling regime with sufficient replication to detect < 20% effect size would have sufficient statistical power to detect between-survey changes as significant changes over time.

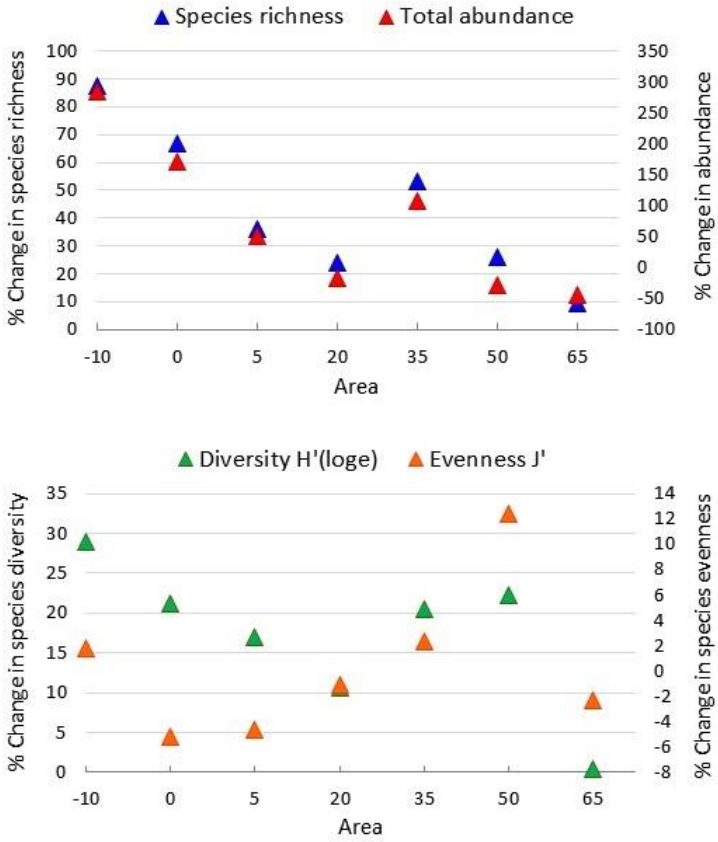


Figure 53. EMB: variability in average species richness, abundance, evenness and diversity (after pooling) between 1999 and 2016, within for each area (-10, 0, 5, 20, 35, 50, 65), based on all available data for each year.

5.0 DIFFERENCES IN FAUNAL ASSEMBLAGES BETWEEN ROEBUCK BAY AND EIGHTY MILE BEACH

RB supported significantly higher average species richness, diversity and evenness than EMB, but significantly lower average abundance (Table 20). However, as expected, these differences were not consistent among individual areas (Tukey's *post hoc* tests, not shown). For example, average species richness for each of SB and OT was not significantly different to any of the EMB areas ($p < 0.05$), while average species abundance for MA was significantly higher than all EMB and RB areas. Diversity and evenness were highly variable among individual areas, though most RB areas (except MA) supported higher diversity and evenness than EMB areas.

Table 20. One-factor ANOVA testing for significant ($p < 0.05$) differences in species metrics (all $\log_{10}$ transformed) between RB and EMB, after pooling. Average values (± 95% CI) for untransformed data are shown.

Source	Variable	ANOVA (main effects)				Average (± 95% CI)	
		df	MS	F	*p*	RB	EMB
Region	*S*	1	2.437	72.361	<0.001	20.9 (1.92)	12.7 (0.51)
	N	1	0.727	6.190	0.013	79.4 (28.0)	82.5 (18.8)
	J'	1	0.475	36.385	<0.001	0.84 (0.02)	0.70 (0.02)
	H' (log$_e$)	1	1.500	86.088	<0.001	2.43 (0.10)	1.75 (0.06)

There was also a significant difference in species assemblages between RB and EMB (one-factor PERMANOVA, Pseudo-F = 10.54, $p = 0.0001$) and, again, pairwise tests (not shown) indicated the difference was consistent for all sub-areas ($p < 0.007$). Average similarity (Bray-Curtis) between RB and EMB was only 20%. mMDS ordinations on distances among centroids for areas and sub-areas supported the PERMANOVA results. The ordination plots (Figure 54a-b) clearly show the separation of the two regions and the significantly greater dispersion of RB sub-areas compared to EMB sub-areas (PERMDISP, t = 13.50, $p = 0.001$), signifying greater heterogeneity in species assemblages within RB. A north-south gradient is also obvious across the EMB areas (Figure 54b).

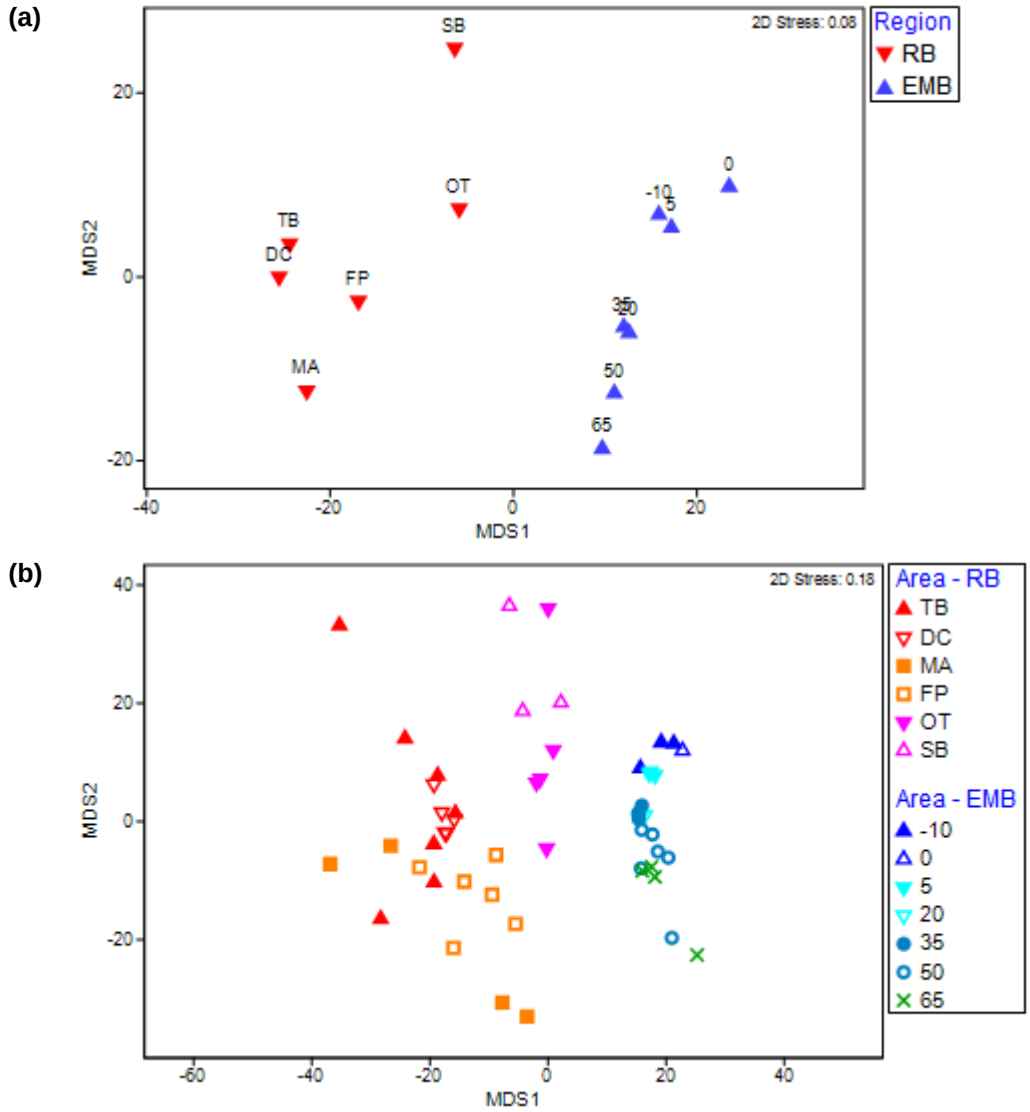


Figure 54. RB 2016 versus EMB 2016: mMDS ordinations of distances among centroids for (a) areas, and (b) sub-areas, based on Bray-Curtis similarity (4^{th} root transformed abundance). Optimum solutions in 3D with stress = 0.06 (a), and 0.12 (b).

6.0 TAXONOMIC LEVEL AND DATA TRANSFORMATION

OTUs rather than species-level taxonomy are used for AnnRoeBIM for ease of identification in the field, budget and time constraints for experts to identify to species-level in the laboratory, and for the fact that published taxonomy is not confirmed for all taxa. Average pairwise Bray-Curtis similarity matrices were generated for *a priori* areas to examine the degree to which taxonomic aggradation might affect estimates of similarity in fauna assemblages within RB and EMB.

Second stage mMDS ordination showed taxonomic level had a much more marked effect on taxa assemblage data than did transformation (Figure 55). Fourth-root (4^{th} root) and presence-absence (p-a) transformations were very similar in their effect, especially for species and family-order levels. Effect of taxonomic aggradation was noticeably greater for class-phylum level than for family-order level, and this effect was greater with a more severe transformation, *i.e*. p-a for family-order level data, and both 4^{th}-root and p-a for class-phylum level data. Class-phyla level data resulted in much higher (almost double) percent similarity between areas than either family-order or species-level data indicating the 'simplification' of the assemblage composition information, making all areas similar, and thereby loosing spatial (and also likely temporal) distinction (Table 21).

This is further illustrated in the ordination plots for distance among centroids for the sub-areas (Figures 56 & 57), where sub-areas can be seen to group somewhat closer together (*i.e.* distance among centroids is smaller) when Class-Phyla level data are used, but are far more dispersed when family-order or species level data are used, and particularly so for EMB. The patterns of dispersion are very similar for family-order and species level data for both regions (Figure 56 & 57). That fact that the plots don't show a closer grouping of sub-areas based on Class-Phyla data despite the much higher similarity values, is partly down to the ability of the ordination to accurately represent the data as a 2- or 3-dimensional plot. In this instance, the 3-dimensional plots provided no additional information and so for visual clarity only the 2-dimensional plots are shown. PERMANOVA pairwise tests found significant differences ($p < 0.011$) among all RB areas regardless of the taxonomic level used. For EMB however, significant differences between areas -10 and 5, and 50 and 65 that were apparent in pairwise tests using 'species' and family-order data ($p \leq 0.05$) were lost when taxa were aggregated to class-phyla ($p > 0.37$).

Thus, there appears to be little loss of resolution for detecting spatio-temporal differences between current OTU taxonomic resolution and family-order level resolution, as the two levels are almost the same. There would therefore be minimal cost saving in identifying to Family-Order only. However, there is a loss of information for detecting change when Class-Phyla level is used.

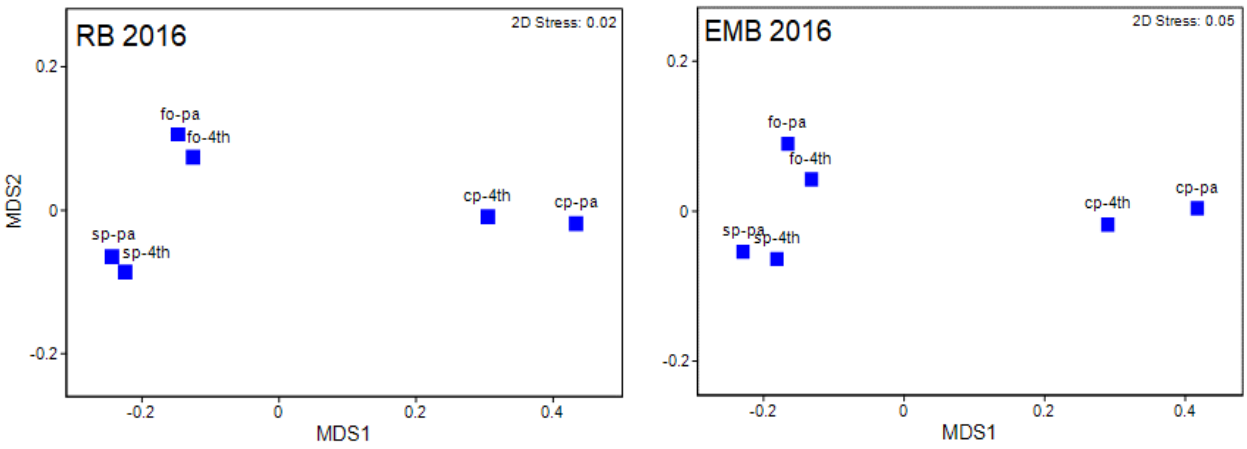


Figure 55. Second stage mMDS ordination (Bray-Curtis similarity) on abundance data, comparing effect of differing data transformations and taxonomic levels; 4th = 4^{th} root transformation, pa = presence-absence transformation, sp = 'species' level, fo = family-order level, cp = class-phyla level.

Table 21. Matrices for average similarity (Bray-Curtis) in faunal assemblages (4th root transformed abundance) within and among areas for RB (TB, DC, MA, FP, SB) and EMB (-10, 0, 5, 20, 35, 50, 65) for differing taxonomic levels.

(a) RB 2016

'Species'

	TB	DC	MA	FP	OT	SB
TB	28.4					
DC	26.0	28.5				
MA	24.2	25.0	31.3			
FP	26.0	24.8	26.9	32.2		
OT	14.8	13.4	15.7	19.0	19.4	
SB	16.7	15.6	14.8	16.7	18.2	31.5

Family-Order

TB	41.4					
DC	39.3	42.3				
MA	38.4	38.7	45.2			
FP	40.5	38.9	38.6	46.2		
OT	26.7	25.6	25.6	30.9	30.8	
SB	28.6	28.6	23.8	30.0	28.9	40.7

Class-Phyla

TB	71.3					
DC	72.3	74.9				
MA	67.3	68.6	69.9			
FP	69.4	68.7	66.0	71.5		
OT	59.2	58.2	56.7	59.0	59.2	
SB	68.5	69.6	62.0	64.2	61.4	73.0

(b) EMB 2016

'Species'

	-10	0	5	20	35	50	65
-10	39.7						
0	45.4	51.7					
5	43.0	49.0	48.5				
20	34.1	36.5	39.6	42.3			
35	33.6	35.9	38.1	39.7	41.0		
50	29.8	31.6	34.3	39.7	39.1	43.7	
65	26.7	29.1	30.5	35.0	32.9	40.9	43.4

Family-Order

-10	46.0						
0	50.7	55.9					
5	49.5	55.2	55.6				
20	40.0	41.1	46.6	49.3			
35	38.3	38.5	44.2	45.2	44.5		
50	35.9	35.1	42.7	46.1	43.9	49.3	
65	34.4	33.7	40.4	42.4	38.6	47.5	49.9

Class-Phyla

-10	72.6						
0	74.6	75.7					
5	72.4	74.6	73.6				
20	63.6	66.5	66.7	66.2			
35	65.9	67.8	68.0	65.2	66.3		
50	66.6	69.1	67.3	64.8	65.2	67.7	
65	68.1	70.3	68.7	64.2	64.7	68.1	69.2

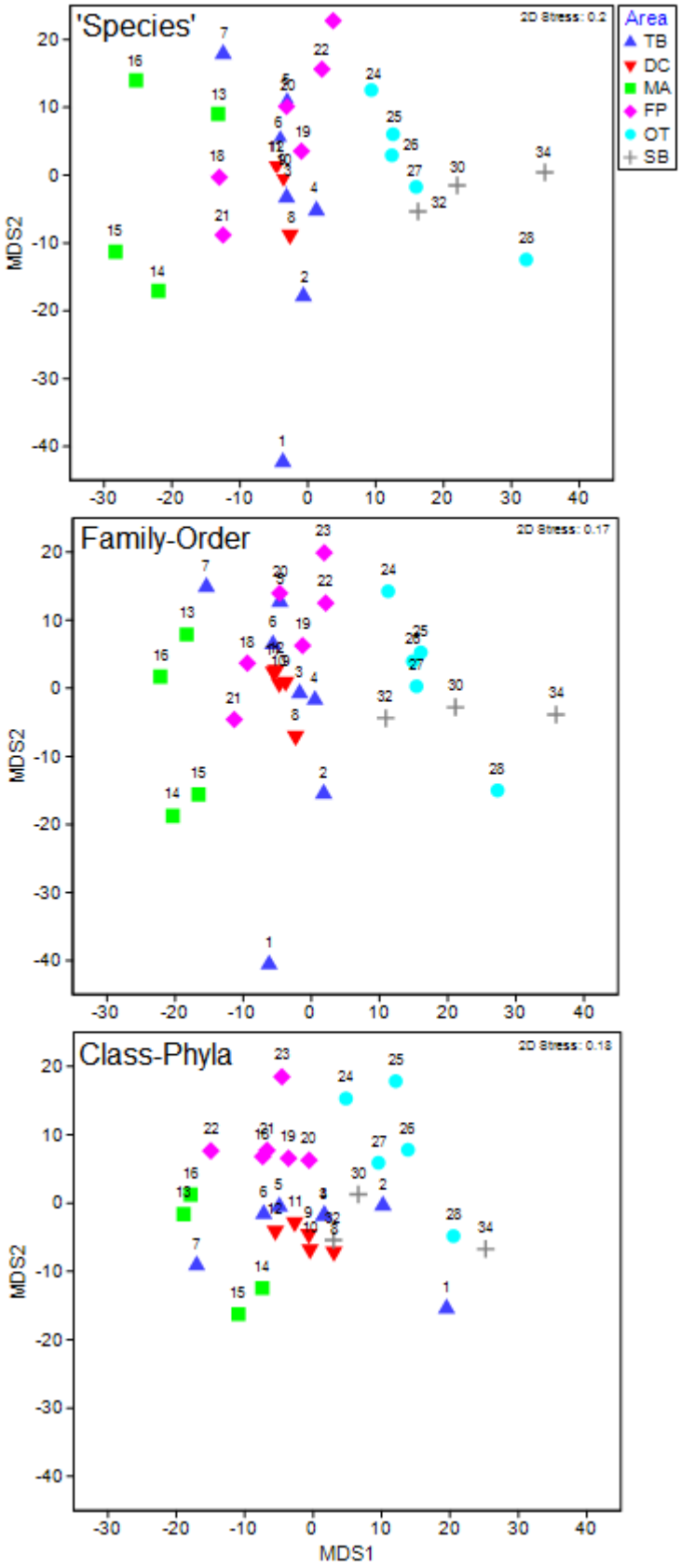


Figure 56. RBB 2016: comparison of mMDS ordinations of distances among centroids for sub-areas, based on Bray-Curtis similarity (4^{th} root transformed abundance), for differing levels of taxonomic resolution (after pooling).

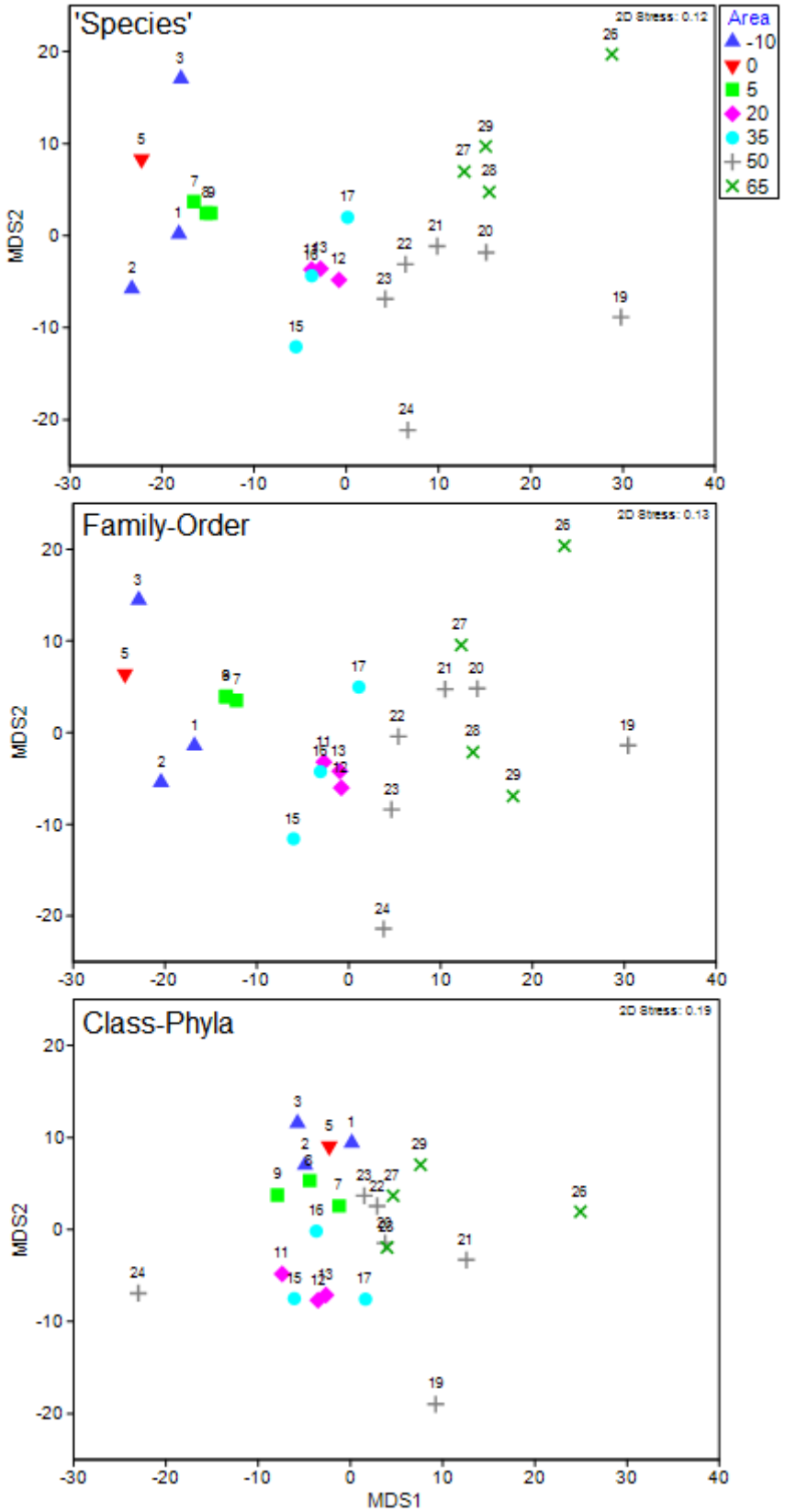


Figure 57. EMB 2016: comparison of mMDS ordinations of distances among centroids for sub-areas, based on Bray-Curtis similarity (4th root transformed abundance), for differing levels of taxonomic resolution (after pooling).

7.0 SHOREBIRD PREY BIOMASS DISTRIBUTION

7.1 Background

RB and EMB are the most important shorebird's areas in Australia and one of the most important non-breeding areas in the East-Asian Australasian Flyway (Rogers *et al.* 2011). The number of shorebirds using RB and EMB exceed 100,000 and 290,000 individuals respectively in the non-breeding season (BirdLife Australia 2018). The importance of these two locations for shorebirds has been linked with the extremely high diversity and biomass of benthic invertebrates. The high abundance and diversity of macroinvertebrates in RB and EMB places these tropical intertidal areas among the richest mudflats in the world (Piersma *et al.* 1998, Compton *et al.* 2008). There are approximately only twelve sites globally where large mudflats rich in shorebirds are found at low tide and, only two are found in tropical areas and RB is one of them (Rogers 2003). Due to the internationally significant number of species they support, RB and EMB were designated as Wetlands of International Importance in 1990 under the Ramsar Convention (1971). In acknowledgment of their ecological value, RB and EMB were recognised as Marine Parks by the State government in 2013 and 2016 respectively.

The distribution of shorebirds in non-breeding habitats is strongly affected by the abundance of intertidal prey (*e.g.* Colwell & Landrum 1993, Finn *et al.* 2008, VanDusen *et al.* 2012). The AnnRoebim database represents an unequal opportunity to evaluate shorebird prey macrobenthic biomass distribution in order to understand shorebirds distribution and habitat use in RB and EMB.

7.2 Methods

The biomass distribution of the main prey of three selected shorebird species was calculated with the most recent data available, the AnnRoebim16 expedition. Shorebird species selected for this analysis included great knots (*Calidris tenuirostris*), bar-tailed godwits (*Limosa lapponica*) and curlew sandpiper (*Calidris ferruginea*).

Distinct steps were followed in order to obtain an accurate mapping of shorebird prey biomass distribution.

1. *Shorebird species*. Shorebird species were selected following three criteria, i) that the species is abundant in RB and/or EMB, ii) there is substantial knowledge on their diet, at least in RB, and/or iii) the species is listed as Critically Endangered under the Environment Protection and Biodiversity Conservation Act 1999 (EPBC Act).

2. *Shorebird benthic invertebrate prey*. There is a critical lack of quantitative data about shorebird diet and prey size selection in RB and EMB. The only available quantitative data for RB is for great knot, red knot (Tulp & de Goeij 1994) and bar-tailed godwit (Estrella 2013). Qualitative data are provided by Rogers (1999, 2006). Where available, complementary information about diet and prey size was obtained from published literature for Australia (Dann 2000) or for climatically similar habitats (Zharikov & Skilleter 2002, 2003, 2004, Finn *et al.* 2008, Estrella *et al.* 2015).

   A number of factors were considered when deciding which benthic macroinvertebrate taxa should be included as potential prey in the diet of each shorebird. Prey selection is subject to prey size limitations in shorebirds. Too large or too small prey can be difficult for shorebirds to capture or swallow, and small-sized prey are often not an energy-efficient option as the energy they provide is too low in relation to the energy spent foraging for them (Zwarts & Wanink 1993, Estrella 2007). Other taxa (*e.g*. Nassariidae) were not considered likely prey as they possess a thick shell which would be difficult for shorebirds to digest (Zharikov & Skilleter 2004, van Gils *et al.* 2005, Quaintenne *et al*. 2010). The bivalve family Lucinidae was also excluded because recent studies

have found that lucinid toxicity constrains selection as prey by red knots (Van Gils *et al*. 2012). Similarly, the polychaete families Amphinomidae ("fireworms") and Phyllodocidae were excluded, as the former are known to possess toxin-coated chaetae, while the latter are suspected to be toxic (Rogers 1999). Consistent with Rogers (1999), tubeworm families Chaetopteridae and Oweniidae were also not included in the diet of shorebirds in RB and EMB.

3. *Benthic macroinvertebrates allometric biomass equations*. To calculate the benthic invertebrate shorebird prey biomass distribution, it is necessary to have access to equations that relate the size (*e.g*. length) of specific taxa to its biomass (ash free dry mass). However, there is a dire paucity of such data for RB and EMB. Since calculation of allometric equations for every potential prey species was beyond the scope of the current project, available equations from published (Rogers 2006, Tulp & de Goeij 1994) and unpublished data (Estrella unpubl.) on RB benthic macroinvertebrates were used, as well as those from published literature for other geographical locations (Drake & Arias 1995, Estrella *et al*. 2015, Choi 2015, Ponti *et al*. 2017, Zwarts & Wanink 1993, Rainer & Wadley 1991, Lovvorn *et al*. 2003). When a specific taxa equation was not found, an existing equation from a morphologically similar taxon was applied. The few instances where the length of an invertebrate was not recorded, the average length from the taxon from RB or EMB was applied.

7.3 Results

7.3.1 Roebuck Bay

In 2016, biomass of main prey species for great knots was concentrated in the northern sections of RB, with highest densities in the Dampier Flats area (Figure 58).

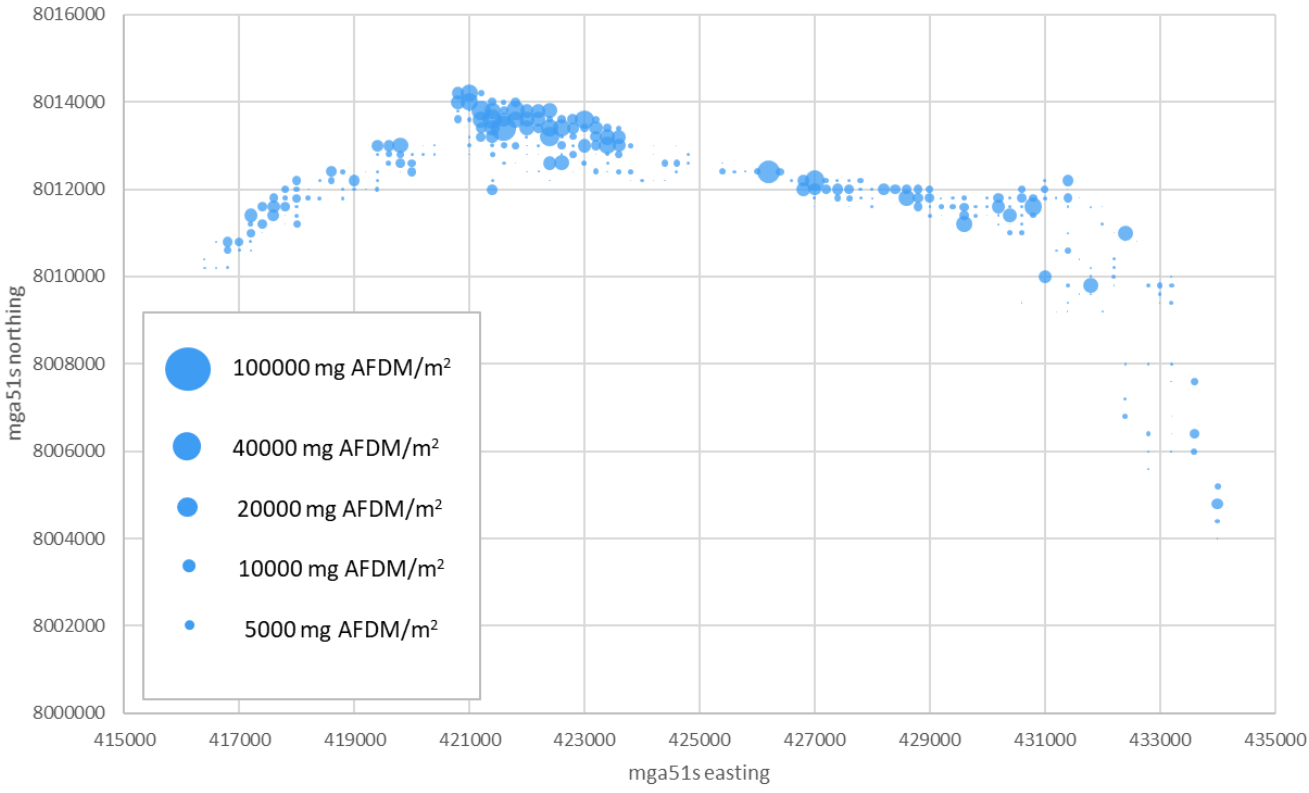


Figure 58. RB 2016: great knot (*Calidris tenuirostris*) main prey biomass density distribution measured in ash-free dry mass per m^2 (AFDM mg/m^2).

Biomass of main prey species for bar-tailed godwits (*Limosa lapponica*) was also concentrated in northern sections of RB, and was typically higher than that of prey for great knots (Figure 59). There were two areas with greatest biomass density for bar-tailed godwit prey; i) Dampier Flats, with higher biomass concentrated offshore, and ii) the intertidal area between Fall Point and One Tree, where higher biomass density was found close to shore (Figure 59). Relatively low biomass densities for both great knot and bar-tailed godwit prey were recorded in the intertidal area near Bush Point.

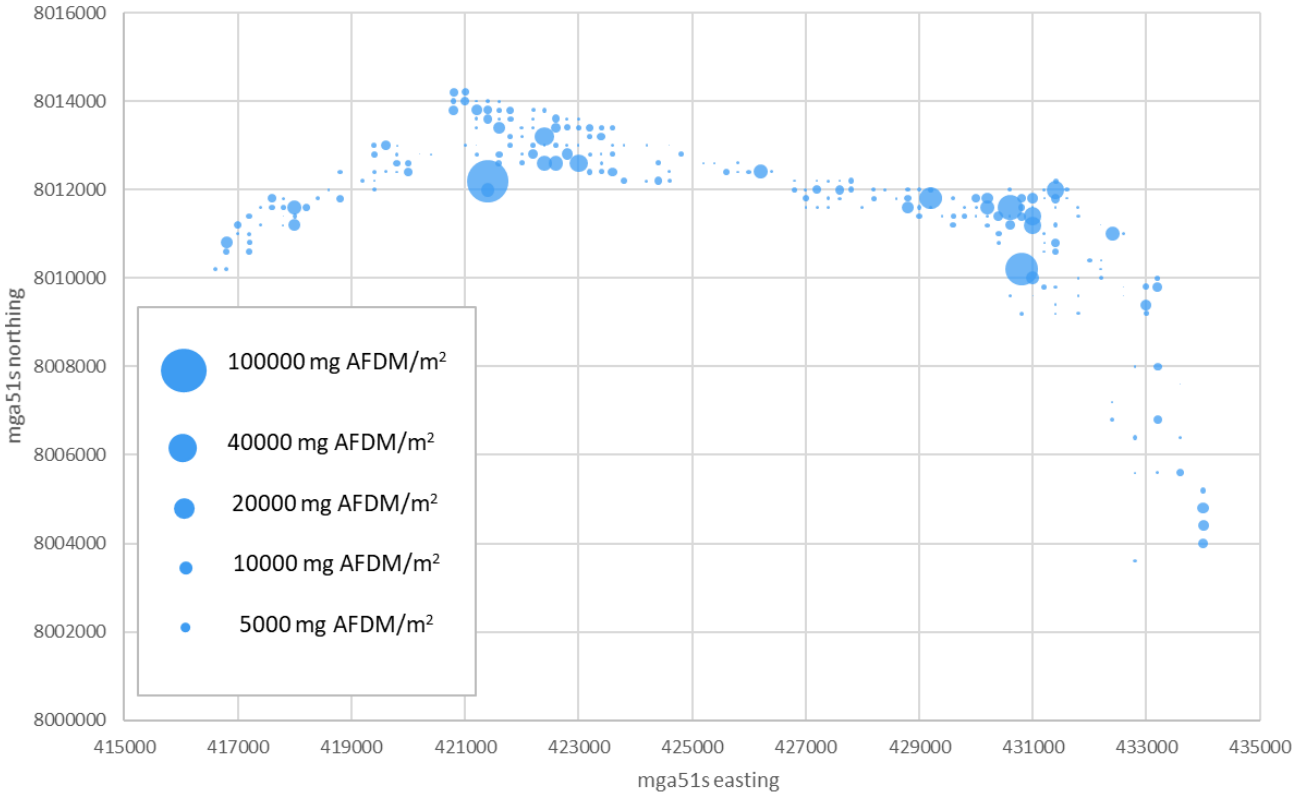


Figure 59. RB 2016: bar-tailed godwit (*Limosa lapponica*) main prey biomass density distribution (AFDM mg/m^2).

Curlew sandpiper prey biomass was more evenly distributed throughout the northern section of RB and to just south of Crab Creek (Figure 60). Highest densities occurred in the north-western section of the bay, where seagrass meadows are abundant, especially in the offshore area of Dampier Flats and the intertidal area of Town Beach. There was also an area of high biomass at Fall Point.

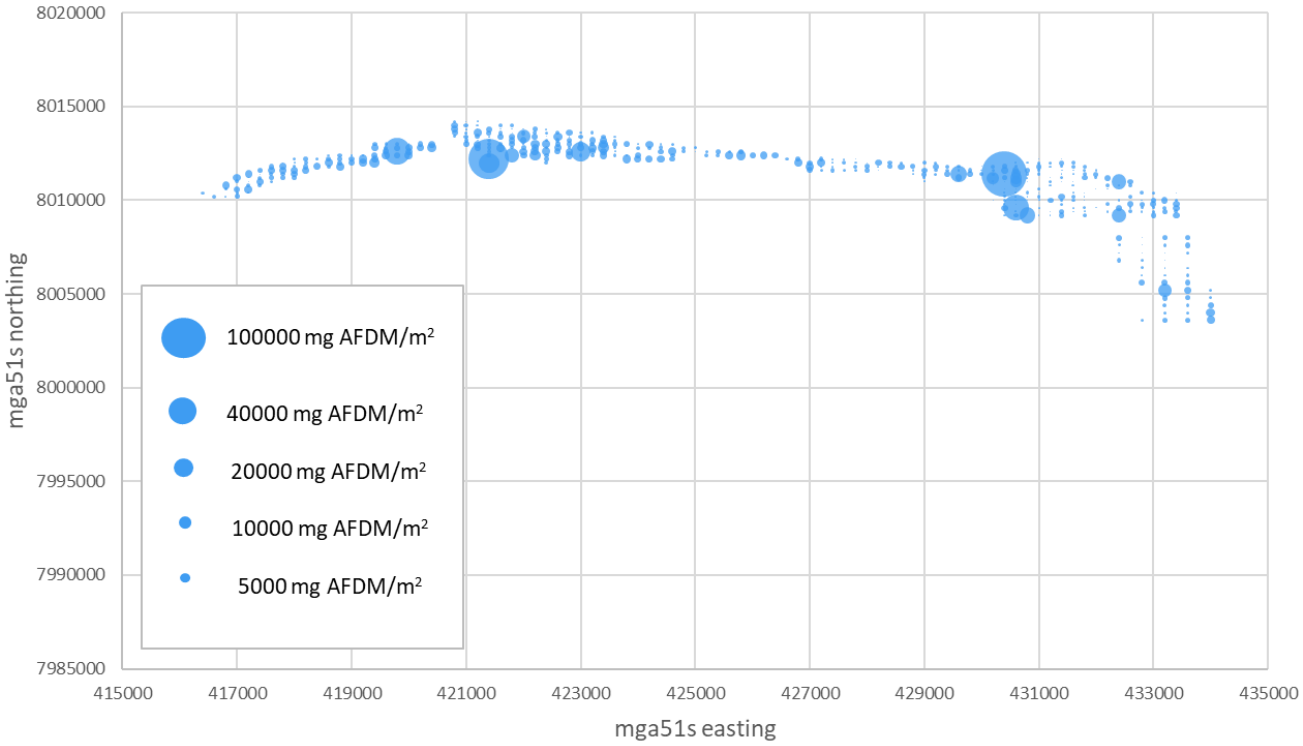


Figure 60. RB 2016: curlew sandpiper (*Calidris ferruginea*) main prey biomass density distribution (AFDM mg/m^2).

7.3.2 Eighty Mile Beach

At EMB, greater density of great knot prey biomass occurrd in ares -10, 0, 5, 50 and 65, than in areas 20 and 30 and 35 (Figure 61). In areas 50 and 65, densities tended to be greater offshore, while in area 5, biomass densities were relately greater both offshore and close to shore, compared to middle flats (Figure 62). Irregular distributions were recorded in other areas (Figure 62).

For bar-tailed godwits, greater prey biomass densities were found in areas -10, 0, 5 and 35, than areas 20 and 65 (Figure 63). The distribution appeared to be relatively homogeneous, without an apparent tidal pattern (offshore vs. inshore) (Figure 64). Stations with greatest biomass densities were mostly those with sipunculids as potential prey for bar-tailed godwits.

Curlew sandpiper prey biomass also showed relatively homogenous distributions within most areas of the beach sampled (Figure 65). In areas 50 and 65, prey biomass density tended to be greater offshore (Figure 66), and again, this was associated with sipunculids as potential prey.

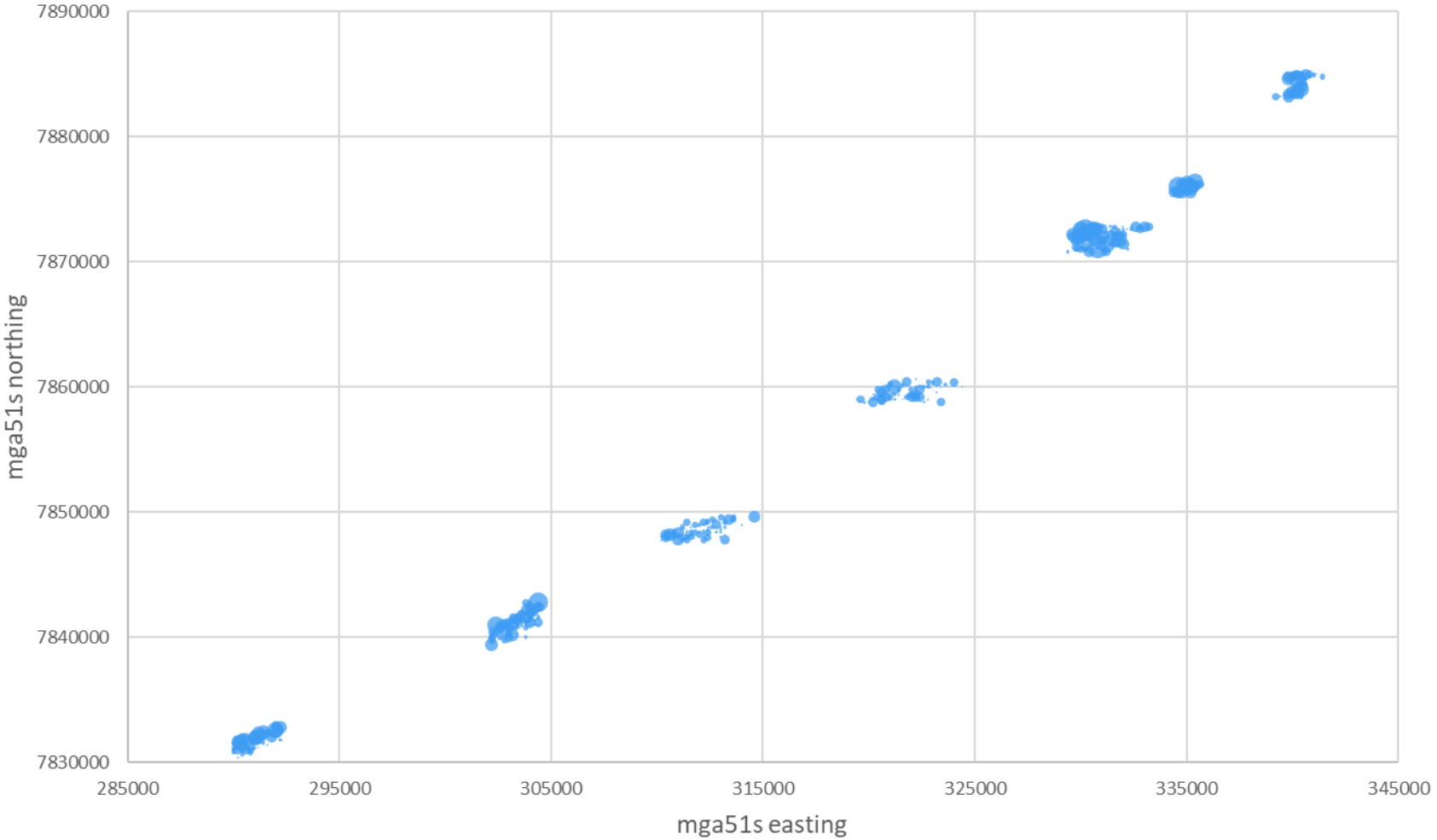


Figure 61. EMB 2016: Great knot (*Calidris tenuirostris*) main prey biomass density distribution (AFDM mg/m^2).

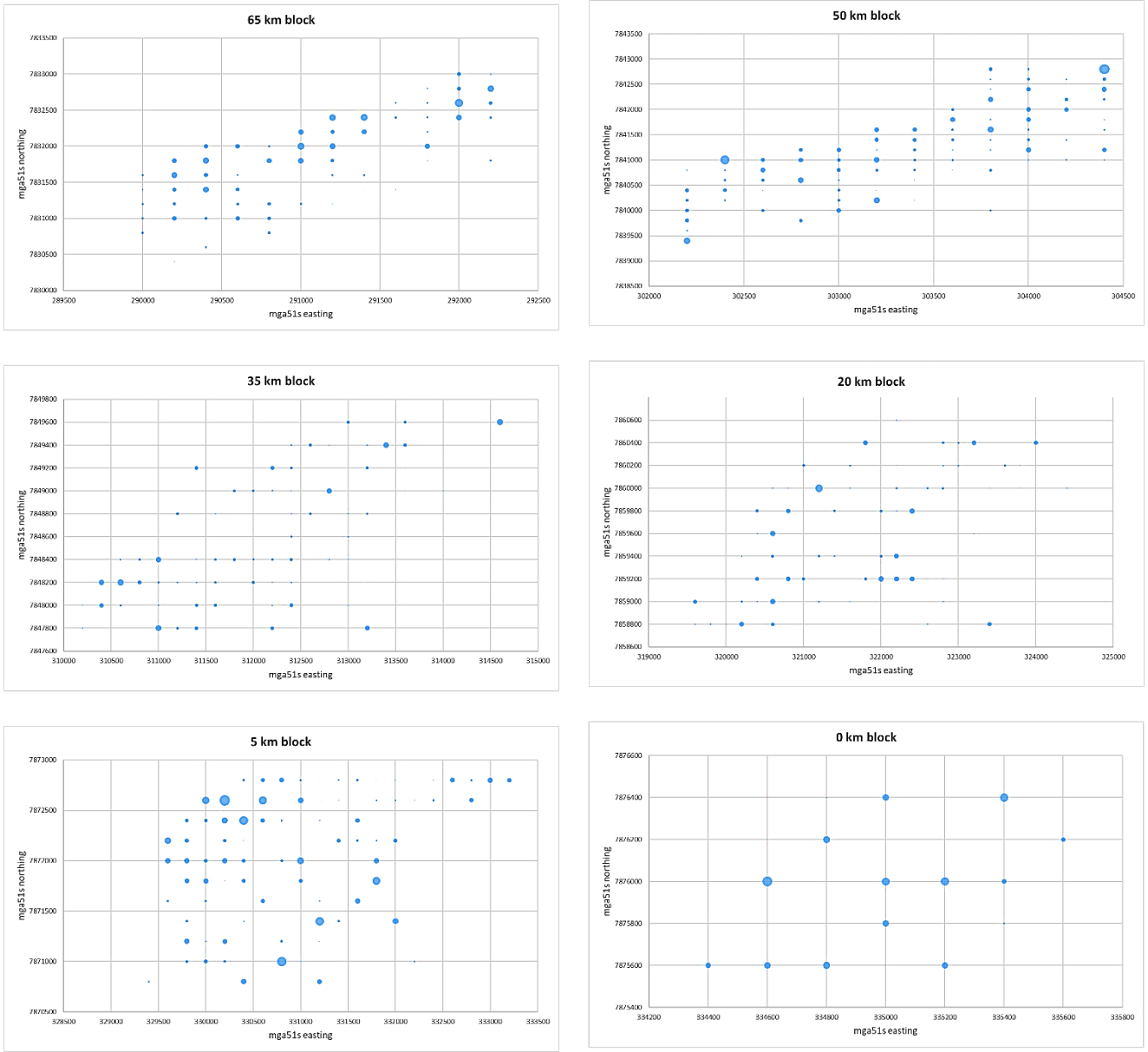


Figure 62. Great Knot prey biomass density (AFDM/m^2) distribution within each area of EMB in 2016.

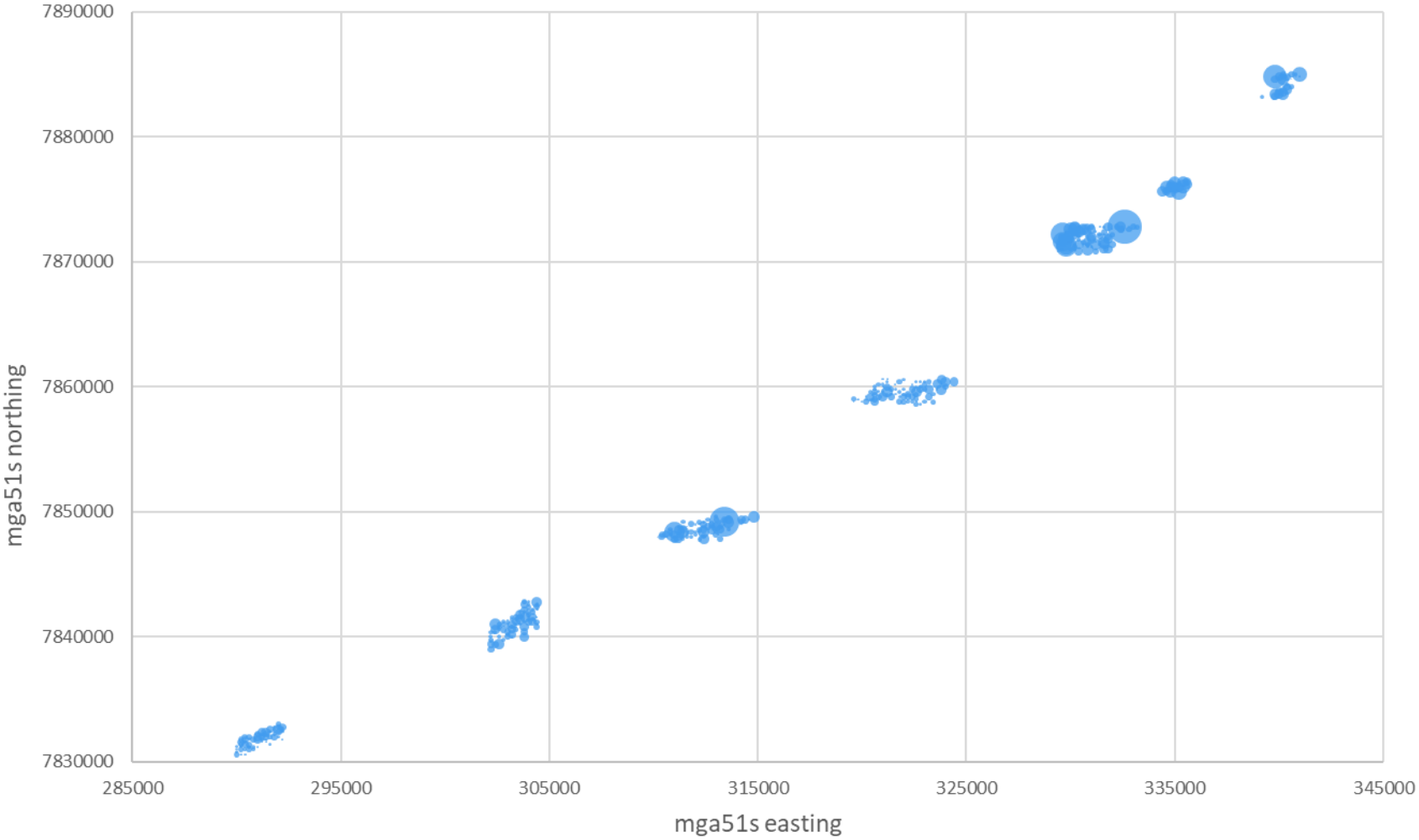
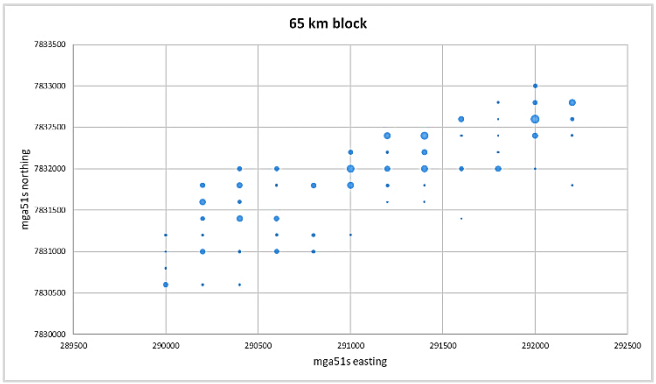
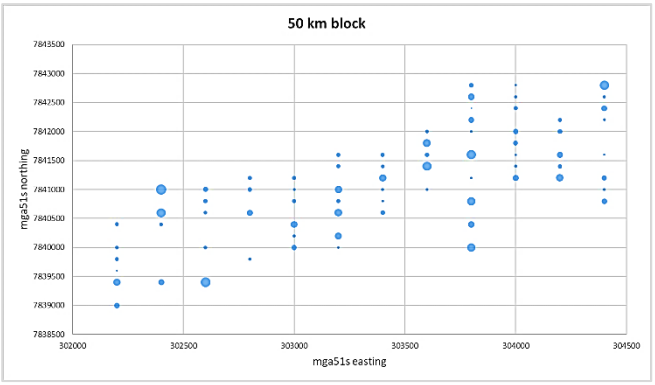
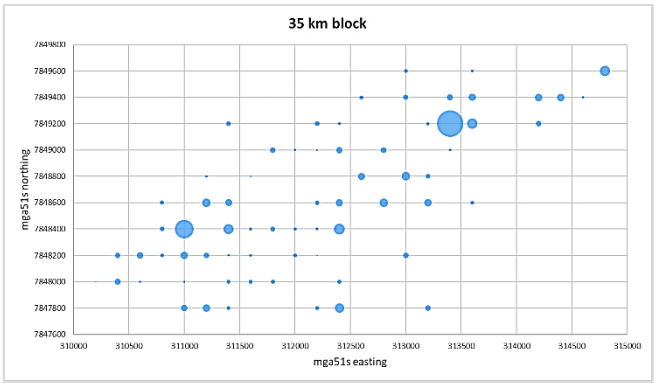
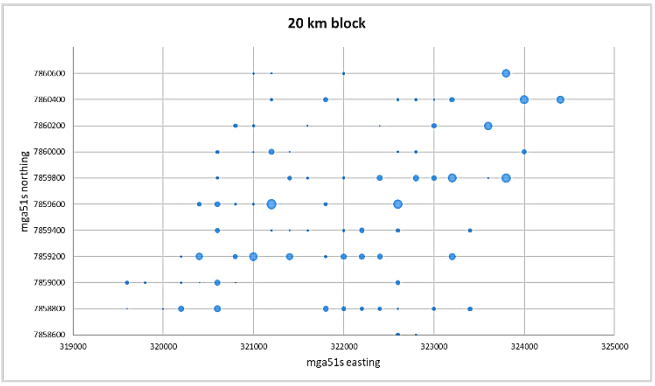


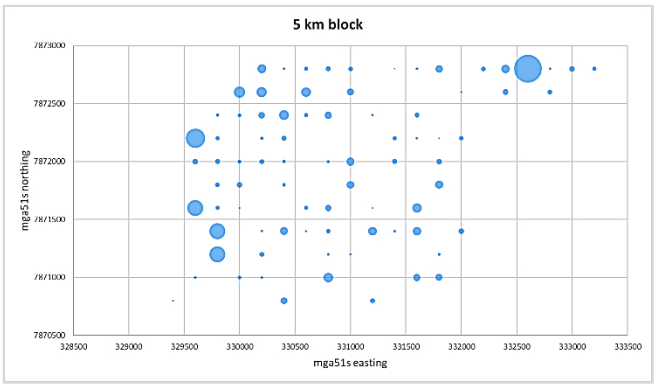
Figure 63. EMB 2016: Bar-tailed godwit (*Limosa lapponica*) main prey biomass density distribution (AFDM mg/m^2).

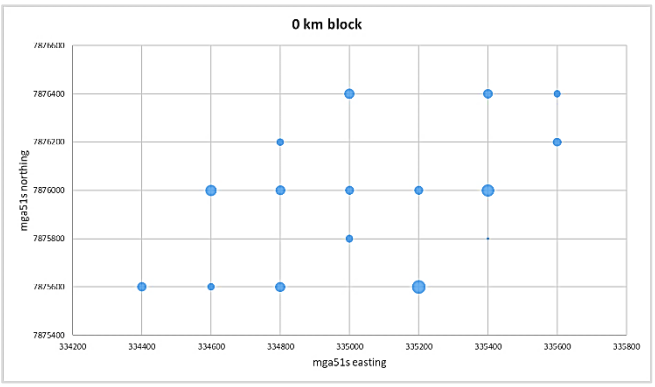












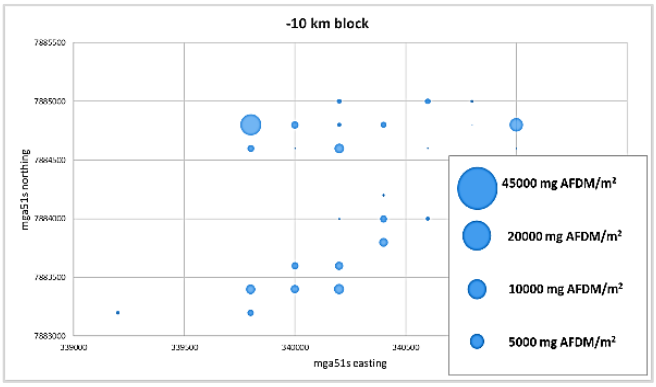


Figure 64. Bar-tailed godwit prey biomass density (AFDM/m^2) distribution within each area of EMB in 2016.

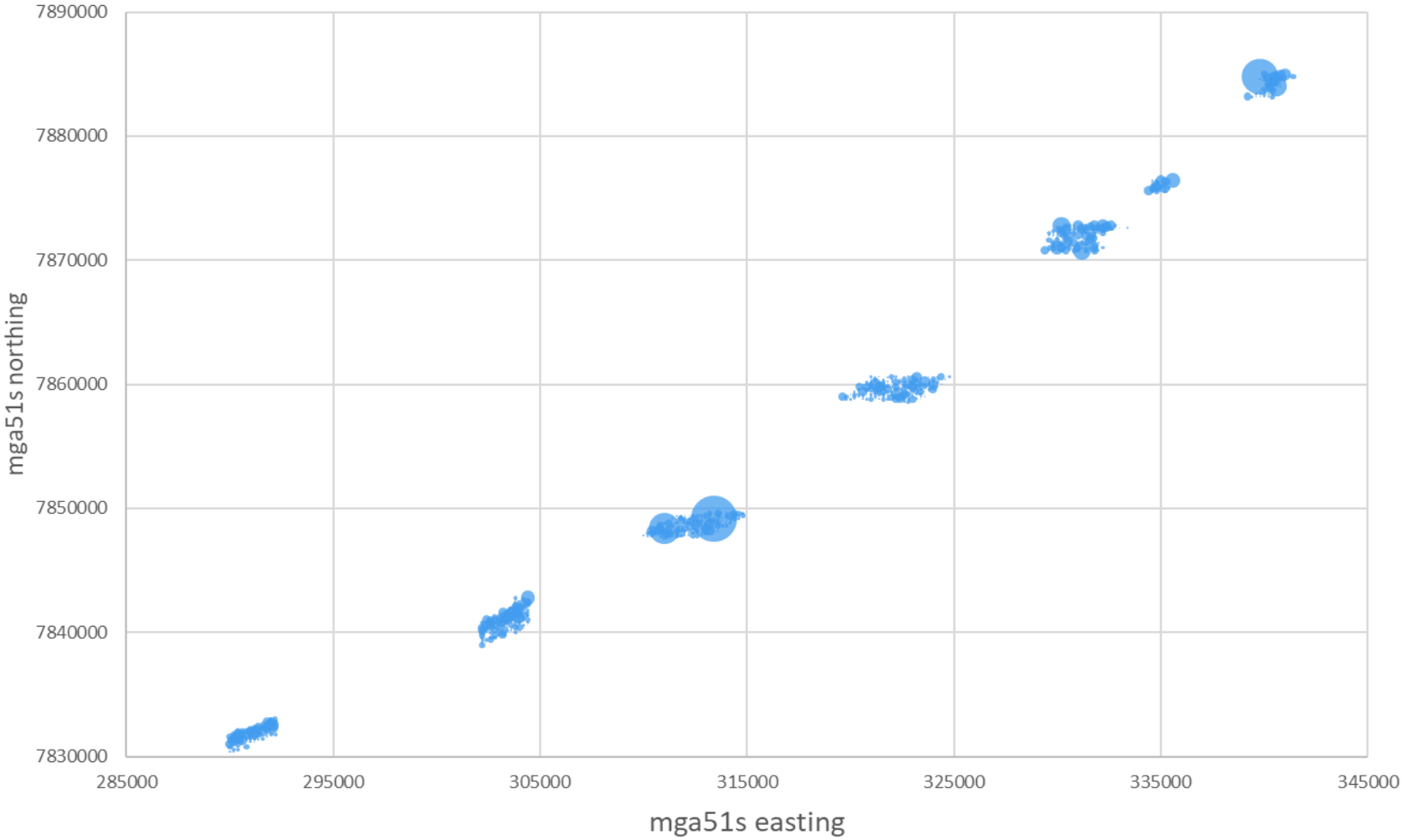
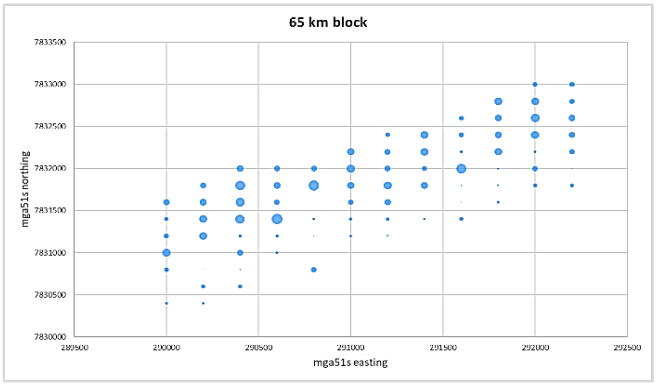
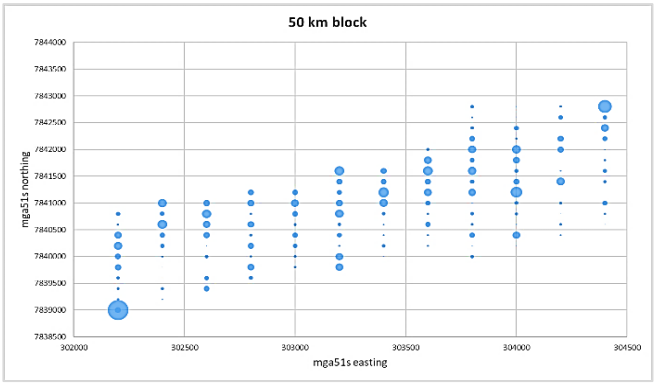
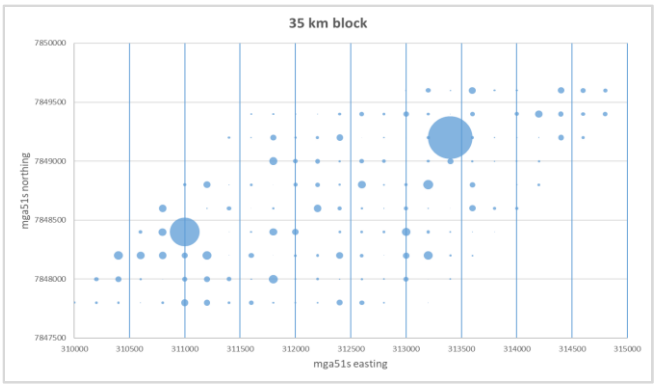
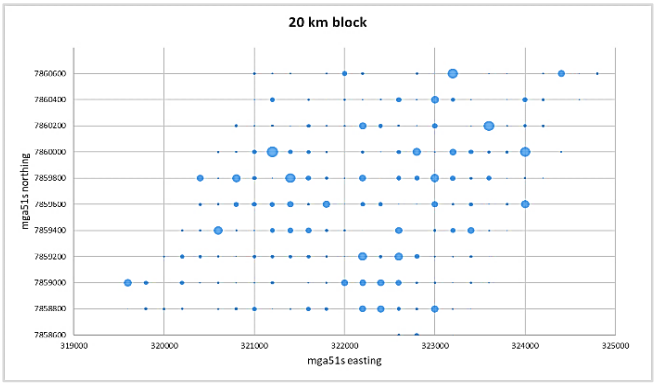


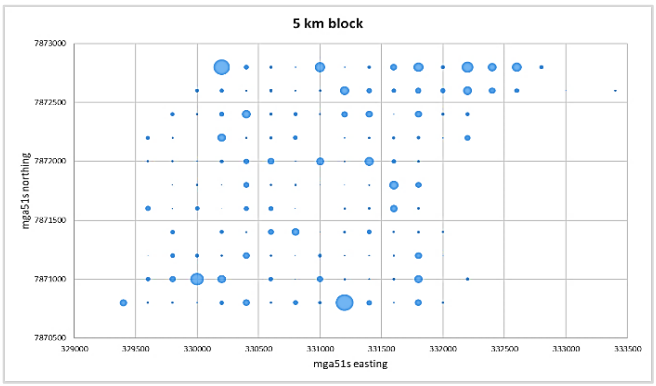
Figure 65. EMB 2016: Curlew sandpiper (*Calidris ferruginea*) main prey biomass density distribution (AFDM mg/m^2).

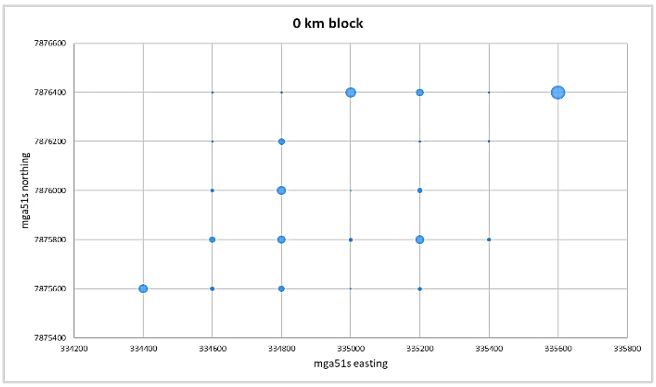












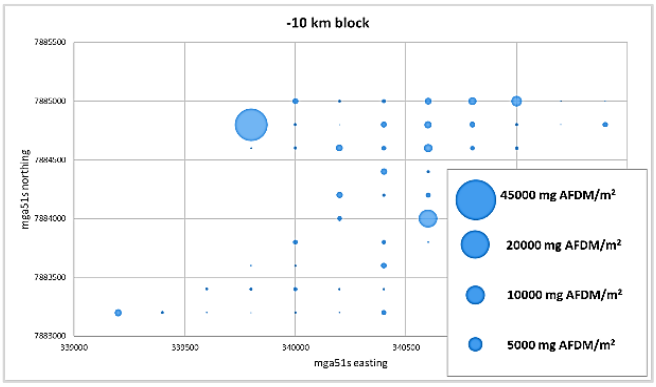


Figure 66. Curlew sandpiper prey biomass density (AFDM/m^2) distribution within each area of EMB in 2016.

7.4 Discussion

There are substantial differences in the prey biomass distribution among the three shorebird species studied (great knot, bar-tailed godwit, curlew sandpiper). In general, the species that appeared to have the highest prey biomass available in RB and EMB was the curlew sandpiper. Curlew sandpipers, are a small-sized shorebird, and are able to feed on smaller prey that may not be energy-efficient or may be too difficult to capture for other medium to large shorebird species (Zwarts & Wanink 1993, Estrella *et al*. 2007). The great knot, which is mainly a molluscivore, has a more restrictive diet (Tulp & de Goeij 1994) than godwits and other sandpipers.

Although the bivalve family Lucinidae was included as a potential prey for shorebirds, recent studies have found that lucinid toxicity constrains its selection as prey by red knots (Oudman *et al.* 2014, van Gils *et al.* 2013). Therefore, it is possible that lucinid bivalves do not play a major role as prey for shorebirds in RB, where lucinid bivalves represent approximately 26% of all the bivalves. However, in EMB, lucinid bivalves represent nearly 40% of all bivalves present. It is unknown if molluscivorous shorebird species carry out a trade-off prey selection in Eighty Mile Beach as they do in other regions where lucinid bivalves are abundant (Oudman *et al*. 2014, van Gils *et al*. 2013).

Comparing the 2016 prey biomass distribution in RB with that in 1997 (Rogers 1999, 2006), there did not appear to be a substantial change in prey biomass for any of the three shorebird species. In 1997 and 2016, the two areas with the highest prey biomass density for bar-tailed godwits were Dampier Flats and the intertidal area between Fall Point and One Tree (referred to as Kraken Corner by Rogers 1999, 2006). For curlew sandpipers, in 1997 and 2016, the two areas with the highest prey biomass densities were Dampier Flats and Fall Point (Rogers 1999, 2006). For great knots, the area with the highest prey biomass density in 1997 was the intertidal area between Fall Point and Crab Creek (Rogers 1999, 2006), while in 2016, the highest prey biomass density was at Dampier Flats.

The present study offers an approximation of prey biomass density distribution for three species of shorebird, but there are some limitations. It is possible that for some species of shorebird, such as the curlew sandpiper, the present study underestimates the food availability. Several species of small-sized sandpipers are able to feed on microphytobenthos (Elner *et al*. 2005, Kuwae *et al*. 2008, Kuwae *et al.* 2012, Mathot *et al.* 2010) and red-necked stints feed on microphytobenthos in RB (Estrella 2013). However, there is a lack of knowledge on the abundance and biomass of microphytobenthos in RB and EMB.

However, it is equally possible that the present study overestimates prey biomass for each of the three species of shorebird examined. Prey biomass abundance and prey biomass availability are not the same (Zwarts & Wanink 1993). Shorebirds prey or prey biomass availability depends on prey size, prey burying depth and prey digestibility (Zwarts & Wanink 1993, Tulp & de Goeij 1994, Zharikov & Skilleter 2004, van Gils e*t al.* 2005, Quaintenne *et al*. 2010). As discussed in section 6.2, too large or too small prey can be difficult to capture or swallow, and foraging small-sized prey may require significantly greater energy to be spent searching for them, than gained from their consumption (Zwarts & Wanink 1993, Estrella 2007). Low-digestible macroinvertebrates (*e.g*. macroinvertebrates with thick shells), although present, cannot be consumed or are not energy efficient (Zwarts & Wanink 1993, Zharikov & Skilleter 2004, van Gils *et al*. 2005, Quaintenne *et al*. 2010). The depth at which invertebrates are buried in the sediment can also limit their availability as prey for shorebirds (Zwarts a& Wanink 1993, Tulp and de Goeij 1994, Nebel & Thompson 2005, Duijins *et al.* 2012). For benthic macroinvertebrates their burrowing depth is a trade-off between needing to return to the surface to feed, and avoiding predation and desiccation (Zwarts 1986, de Goeij & Luttikhuizen 1998, de Goeij *et al*. 2001, 2008). Macroinvertebrate size influences their burrowing capacity. Large crabs can also burrow out of reach of many shorebird species and are therefore unavailable much of the time. However, large crabs become available when they emerge at the surface to feed. In bivalves, siphon size, which is related to body size, limits the depth to which they can burrow,

as the siphon needs to be long enough to reach the surface to feed and breath (Zwarts & Wanink 1989, de Goeij *et al*. 2001). de Goeij (1999) studied two bivalve species (*Siliqua pulchella* and *Serratina piratica*) that are common prey for great knots and found that in RB, specimens within the ingestible size range, can burrow to a depth of > 44 mm (4.4cm) where great knots cannot reach.

During the AnnRoebim-16 mapping expeditions, all cores were inserted into the sediment to a depth of 20 cm, except where a shell layer was present at a shallower depth. While the samples captured the available food for a wide range of shorebird sizes - from small red-necked stints (ave. bill size 1.8 cm) to large shorebirds like the eastern curlew (bill size 12.8 - 20.1 cm) – they may overestimate the available biomass for small to medium sized shorebirds at other times of year due to the depth of coring. Macroinvertebrate burrowing depth is known to have a seasonal component, at least in temperate areas, and while macroinvertebrates may burrow deep in the sediment in winter, they typically remain close to the surface in summer (Zwarts & Wanink, 1993). In sampling to a maximum 20 cm depth, it is therefore assumed that biomass captured is broadly representative of that present at different times of year.

Notwithstanding the above limitations, the available data are considered to offer a good approximation of the potential available prey biomass for shorebirds. From a management point of view, the study offers some significant information, especially for RB where human disturbance is an issue of concern for shorebird conservation in the area (Rogers *et al*. 2006, Sitters *et al.* 2012). While only three species of shorebirds have been included in the current analysis, they feed on prey of differing species and size, and therefore they exemplify a broad range of shorebirds predators. For all three species, the area that supports the greatest biomass density in RB is the intertidal area of the norther section of the bay, from the Port of Broome to Crab Creek. This section is also where highest human interaction occurs (Rogers *et al.* 2006, Sitters *et al.* 2012). Any disturbance that negatively effects the food resources of shorebirds, or disrupts or prevents shorebird feeding in this section of the bay may have a substantial negative effect on the entire RB population, since this section appears to support uniquely high prey biomass density. No other suitable feeding grounds with similarly high prey biomass density are known to occur. The intertidal area between Crab Creek and Bush Point may offer one such alternative, however there are no recent data to evaluate the quality of this area as a feeding ground for shorebirds.

In conclusion, the potential available prey biomass for three important species of shorebird in RB and EMB have been detailed. These three species are those for which the most dietary information are available. Although there are many other abundant shorebirds at RB and EMB, it was not possible to conduct a similar analysis on these species because of a lack of information (published or unpublished) on their diet, prey size selection and most importantly, the allometric equations to calculate the biomass of macroinvertebrates. The current analysis demonstrates the importance of the northern beaches at RB for these three species, and undoubtedly, if the relevant data were available, the same area would likely be important for other species also. Data on foraging behaviour of satellite-tagged birds during low water also tends to support the importance of the northern beaches, but these data purely show where the birds are present, and does not provide any indication on whether feeding, or what prey items they may be using.

7.4.1 Knowledge Gaps and Recommendations

- Obtain quantitative data about shorebird diet and prey size selection in RB and EMB.
- Develop benthic macroinvertebrates allometric biomass equations for RB and EMB, at least for the most abundant taxa and the main shorebird prey.
- Evaluate if foraging shorebirds in the northern section of RB are subject to human disturbance.
- Evaluate macroinvertebrate abundance and prey availability for shorebirds in other sections of RB not examined in this study *e.g*. Crab Creek to Bush Point.

8.0 MONITORING PROGRAM FOR ROEBUCK BAY AND EIGHTY MILE BEACH

8.1 When to Sample

Modelling by Compton (2017), using MonRoeb data (1996 - 2005), demonstrated there was clear seasonality in benthic macroinvertebrates communities in RB. For on-going AnnRoeBIM monitoring, it is therefore recommended that sampling is standardised to the same time each year. Ideally, sampling should be conducted in spring when access to the mudflats is easiest and most migratory shorebirds have arrived, thus ensuring data collected is representative of communities important to the shorebirds. However, sampling should not be left too late in the season, such that shorebird feeding pressure has significantly reduced macroinvertebrate abundances and densities.

8.2 Where to Sample

The current analyses demonstrate a range of spatial response in benthic inter-tidal macroinvertebrates of RB and EMB:

- In RB, there is a change in fauna composition around the bay, from Town Beach to Southern Beach, while at EMB, composition changes from north to south along the beach. Continued monitoring in different areas of each region is therefore recommended in order to adequately capture these spatial gradations:
    - **Six areas in RB**, *i.e.* TB, DC, MA, FP, OT and SB;
    - **Seven areas at EMB**, *i.e.* -10, 0, 5, 20, 35, 20 and 65 km blocks.
- Depending on budget, some sampling areas (*i.e.* every second area) could be dropped, but maintaining the overall geographic spread in each region is important.
- The effects of inundation, penetrability and seagrass cover need to be considered. Although each appears to have only a small influence on whole community structure (< 10%), they have a statistically significant influence on species assemblages within individual areas. The poor predictive capability for environmental data observed in the current analyses, in part reflects the 3-core sampling strategy, and pooling introduces more variability through the use of average values for environmental data in statistical analyses. However, this was unavoidable. The expectation is that with greater sampling intensity (*i.e*. 9 cores at each site -see section 8.3 below) and site-specific environmental data will likely improve predictive capability.
    - To control for **inundation**, for ongoing monitoring we therefore recommend stratifying sampling to only monitoring within the mid (~20-80%) inundation zones of each region;
    - To control for sediment grain size, we recommend continuing to measure **penetrability** for use as a covariable in univariate and multivariate analyses of fauna data. It is not recommended that the program target specific penetrability zones as the zones change by location around the bay and will also likely change in response to cyclones;
    - To control for **seagrass (and algal) cover**, we recommend continuing to measure relative cover for use as a factor in univariate and multivariate analyses of fauna data.

8.3 Number of Stations and Number of Cores per Station

The pronounced variability in the raw datasets of individual samples shows that the sampling size of 3 cores/sample (total surface area = 0.025 m^2) is too small to be informative of the species assemblages present, and is also problematic for robust univariate and multivariate analysis of spatial and temporal change. The current method shows where species are present, but the absence of a taxon does not necessarily show it is not present, as opposed to not being sampled due to the small sample size. For both RB and EMB, we therefore recommend:

- A minimum sampling size of 9 cores per sample (total surface area = 0.075 m^2);
- A minimum 15 to 20 replicate samples per area, to provide sufficient statistical power to detect a 20% change.

8.4 Indicators and Guideline Values

Four structural diversity measures are recommended as indicators in a weight-of-evidence approach for ongoing monitoring of intertidal benthic macroinvertebrates in RB and EMB. The indicators include univariate measures of total species richness (S), abundance (N) and Shannon Weiner diversity (*H'* $\log_e$), and multivariate Bray-Curtis (B-C) similarity that measures change in whole assemblage composition. These indicators are listed in Table 22. While other commonly used diversity measures were examined in the current report, these were either strongly correlated with the selected indices (*i.e*. Margalef's index and Simpson's index), or appeared less responsive to the spatial and temporal gradations in habitat variables (*i.e*. Pielou's evenness index).

The *Australian and New Zealand Guidelines for Fresh and Marine Water Quality* (ANZG 2018) has established a framework for monitoring aquatic ecosystems in Australia, based on the use of site-specific guideline values (SSGVs) in a multiple lines of evidence approach. SSGVs are usually set for abiotic and biotic indicators in terms of some quantum of change from 'reference' condition, with the extent of allowable change sufficiently small as to minimise risk of 'significant' disturbance to the ecosystem. ANZG (2018) has set the Australian standard for developing such targets, whereby the guideline values for indicators are set at the 20th percentile (when a lower limit is needed) and 80th percentile (when an upper limit is needed) of the reference condition. The 20th and 80th percentiles are deemed to be approximately equivalent to ± one standard deviation around the median (50th percentile), and it is argued that this level of change is unlikely to signify a high level of disturbance to the ecosystem (ANZG 2018).

Consistent with ANZG (2018), the 20th percentile (20%ile) values of pooled samples (9 cores/sample) from AnnRoeBIM-16 were therefore used as SSGVs for the selected indicators (S, N, *H'* $\log_e$ and B-C similarity) for RB and EMB (Table 22). Separate SSGVs were calculated for each area within RB (TB, DC, MA, FP, OT, SB) and EMB (-10, 0, 5, 20, 35, 50, 65). For the purposes of the current assessment, the SSGV for whole species assemblages was calculated as the 20%ile value of average pairwise similarity (B-C) amongst pooled samples within each area.

In order that SSGVs derived from pooled samples better represented the mid inundation zones recommended for monitoring, pooled samples with corresponding average inundation values of < 15% and > 80% were excluded from the calculations. For area MA in RB and area 0 in EMB, this resulted in too few datapoints to have confidence in the SSGVs for these areas (Table 22). It must be emphasised that the SSGVs presented here are not intended to be definitive. Although samples were pooled to avoid as best as possible the confounding effects of inundation and penetrability, pooling data across stations will not necessarily produce comparable data to that from improved replicate sampling at individual stations.

Therefore, SSGVs presented here should be viewed as interim only, and revised once the next round of monitoring using a larger sampling unit (*i.e.* 9 cores/sample) has been completed. The range of indices tested in the current study should also be re-assessed in future years to determine consistency in their response to habitat variability.

The aim for on-going monitoring is to compare the median values from survey data against the SSGVs to determine if there has been a change from the reference (*i.e*. 2016) condition and therefore significant ecological effect. Inherent in the use of the 20%ile of reference data to derive SSGVs is the fact that monitoring (and 2016) data may be less than the SSGV at least 20% of the time. Therefore, a statistical test is required to determine if there has been a statistically significant change from reference condition, as opposed to one or two values exceeding the SSGV (which happens naturally in the reference dataset). The approach requires a minimum amount of monitoring data (typically >10 data points) in order to statistically compare the data to the SSGV using a non-parametric rank test (*e.g*. Wilcoxon signed-rank test). However, where the number of monitoring points is less than 10, then the average may better represent the distribution of the dataset, and using a parametric univariate procedure to test between the SSGV and the mean of the monitoring data (*i.e*. one-tailed t-test) is a robust statistical approach.

Median values below the SSGVs that are also statistically significantly lower than the SSGV or, in the case of Bray-Curtis similarity, statistically lower than the 2016 condition, indicate areas that are significantly altered. Values that are lower than the SSGV but are not statistically lower indicate no (significant) adverse change. Similarly, values higher than the SSGV but not statistically higher indicate no adverse change, and values that are statistically higher than SSGV also indicate no adverse change. The management goal in using the revised SSGVs is to identify and, wherever possible, prevent worsening ecological condition. There is also an assumption that 2016 reflects baseline. It is unknown whether 2016 reflects 'pristine' reference condition, or an existing degree of alteration. This may become apparent once monitoring commences and data on potential contaminants of concern are also collected.

Table 22. Indicators and interim SSGVs for RB and EMB, applicable to 15% to 80% (inclusive) inundation zones.

Region	Area	*Count*	Interim SSGV (= 20%ile of 2016 data)			
			Species Richness	**Total Abundance**	**Shannon Weiner**	**Similarity (B-C)**
RB	**TB**	*18*	15	36	2.4	38%
	DC	*16*	27	44	2.6	44%
	MA	*1*	43	102	3.3	--
	FP	*11*	24	53	2.5	39%
	OT	*21*	8	15	1.6	36%
	SB	*12*	8	17	1.7	44%
EMB	**-10**	*10*	11	66	1.4	40%
	0	*3*	8	57	1.5	50%
	5	*19*	10	85	1.1	38%
	20	*28*	10	36	1.3	37%
	35	*25*	11	58	1.3	36%
	50	*32*	11	31	1.9	38%
	65	*17*	12	53	1.6	45%

Although not examined in the current report, it may also be worthwhile investigating the feasibility and value in using individual species or taxa as indicators for targeted monitoring, such as monitoring for change in shorebird prey biomass and distribution. It was beyond the scope of the current report to investigate individual taxa given the high diversity within the system and unknown relative importance of individual taxa as indicators of system health.

8.5 Budget Matrix

The proposed monitoring design incorporates a range of parameters which may be varied to provide different options in terms of a monitoring program. By reducing aspects such as 1.) number of areas within each system to sample, 2.) number of replicate samples to collect within each area, and 3.) level of taxonomic resolution to use, the cost of the program can be changed to suit an available budget. Obviously changing one or more of the three aspects also affects the sensitivity, resolution and spatial coverage of the final program, and as such there will be a compromise between effectiveness of the design and cost.

A very rough approximation of differences in budget for various combinations of number of sampling areas, number of replicate samples, and taxonomic resolution is presented in Table 23. For this exercise, a nominal sum of $1,000/sample for 'species' and Family-Order level resolution, and $500/sample for Class-Phyla level resolution, was used to represent the total cost to collect, sort and identify taxa and analyse data. The estimated number of replicate samples (9 cores/sample) required to detect a specific effect size was based on power analysis on MonRoeb16 species richness and abundance data for 12 cores/sample (refer section 3.5 Table 10).

As a compromise for budget and logistical constraints, we recommend that fewer areas are sampled but number of replicates maintained, in order to provide sufficient statistical power, rather than reducing the number of replicate samples within each area. It is also recommended that taxonomic resolution is to current OTU or Family-Order level as a minimum, given the loss of information when only Class-Phyla level data are used. The cost saving by going to Family-Order versus current OTU is likely minimal, as many specimens identified to OTU are readily identifiable and distinct, and so time (cost) saving is minimal.

Table 23. Budget matrix for differing sampling designs and taxonomic resolution; nominally $1,000/sample for 'Species' (OTU) and Family-Order, and $500/ sample for Class-Phyla. Matrix is applicable to both RB and EMB.

Taxonomic level	Effect size	No. Replicate Samples	No. of Areas					
			1	2	3	4	5	6
'Species' / Family-Order	10%	30	$30,000	$60,000	$90,000	$120,000	$150,000	$180,000
	20%	15	$15,000	$30,000	$45,000	$60,000	$75,000	$90,000
	30%	8	$8,000	$16,000	$24,000	$32,000	$40,000	$48,000
	40%	3	$3,000	$6,000	$9,000	$12,000	$15,000	$18,000
	50%	3	$3,000	$6,000	$9,000	$12,000	$15,000	$18,000
Class-Phyla	10%	30	$15,000	$30,000	$45,000	$60,000	$75,000	$90,000
	20%	15	$7,500	$15,000	$22,500	$30,000	$37,500	$45,000
	30%	8	$4,000	$8,000	$12,000	$16,000	$20,000	$24,000
	40%	3	$1,500	$3,000	$4,500	$6,000	$7,500	$9,000
	50%	3	$1,500	$3,000	$4,500	$6,000	$7,500	$9,000

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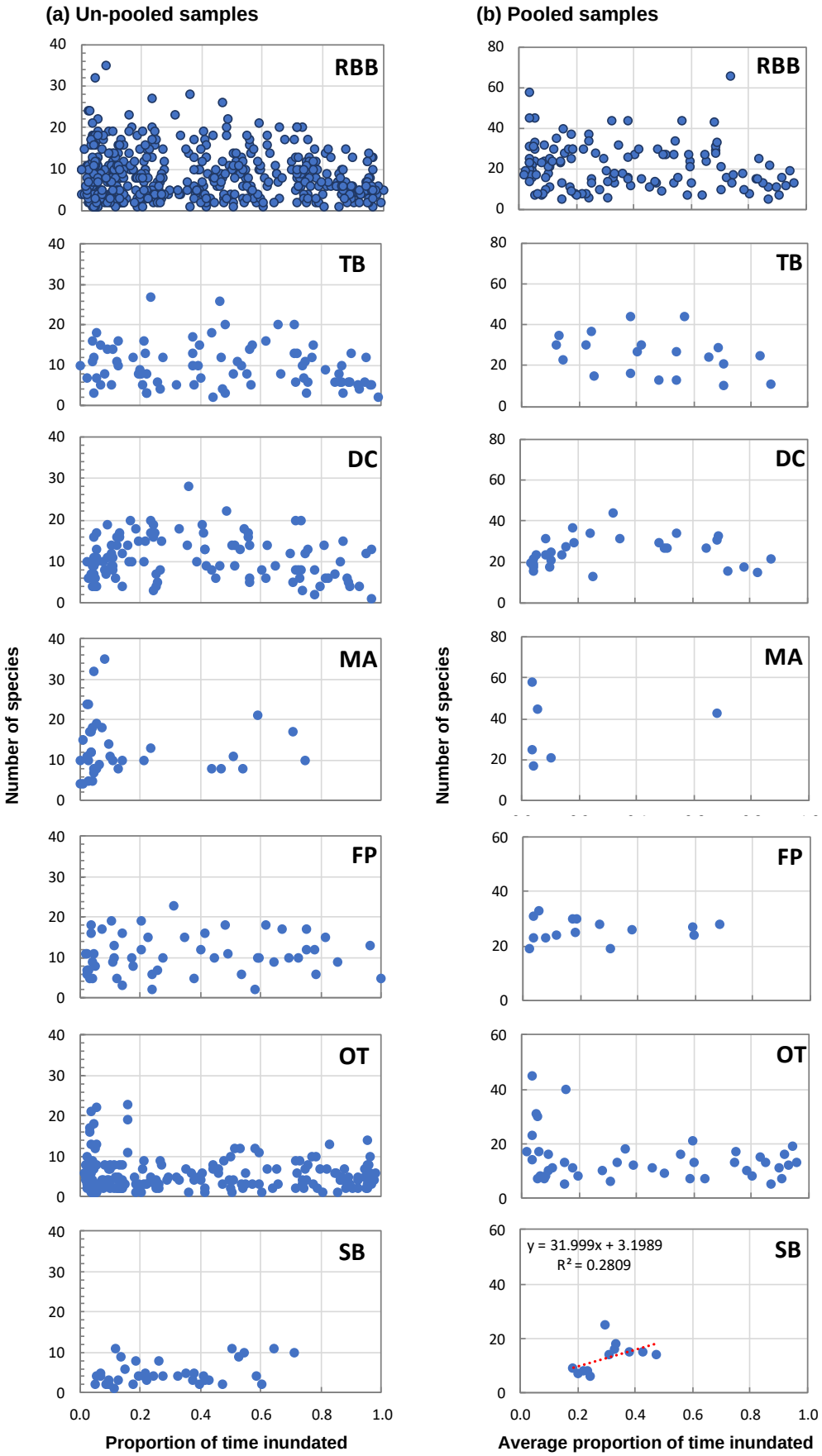
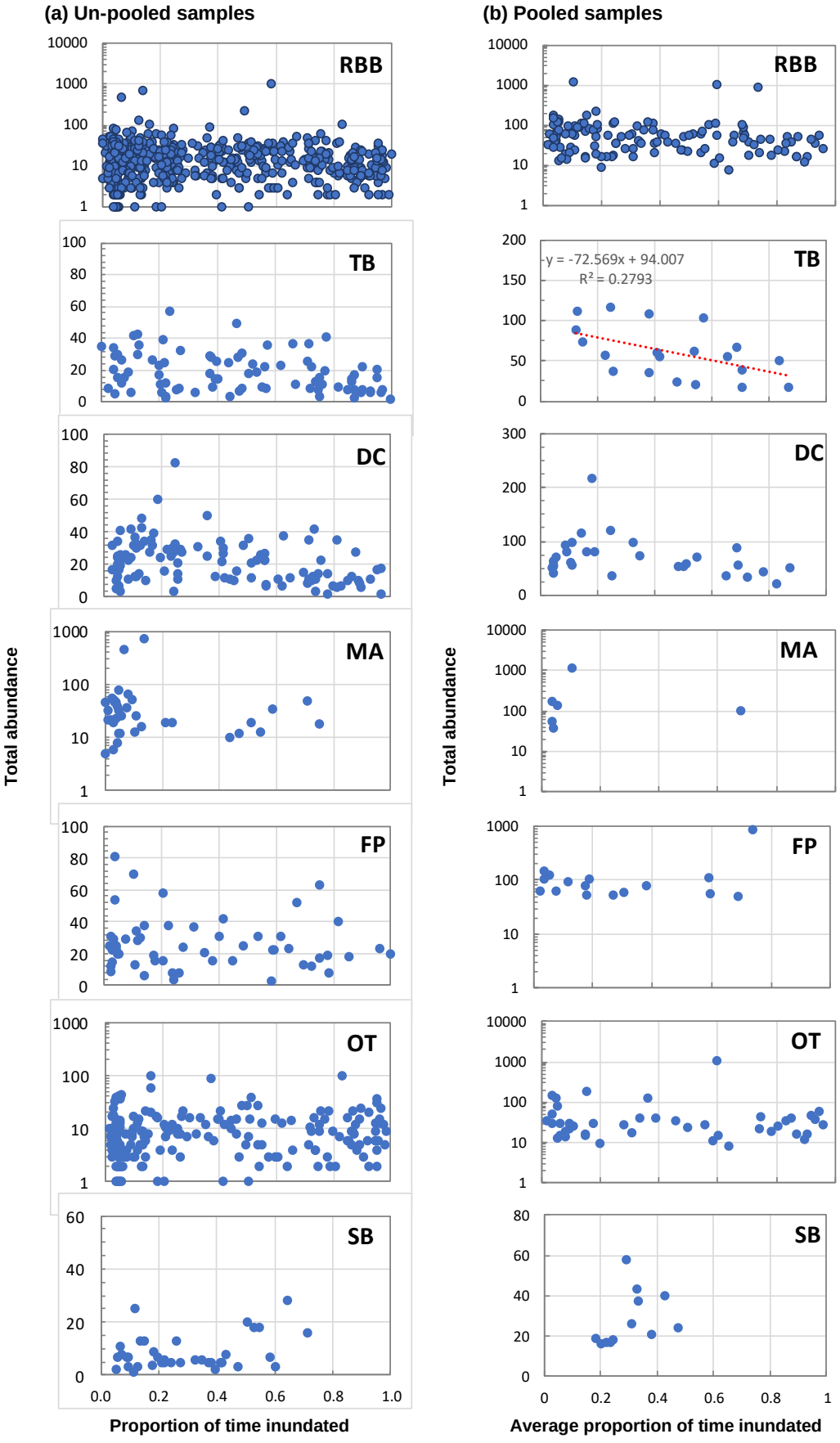
APPENDIX 1. RB 2016 SCATTER PLOTS

Relationships between species metrics and inundation and penetrability

RBB16: Species Richness vs Inundation

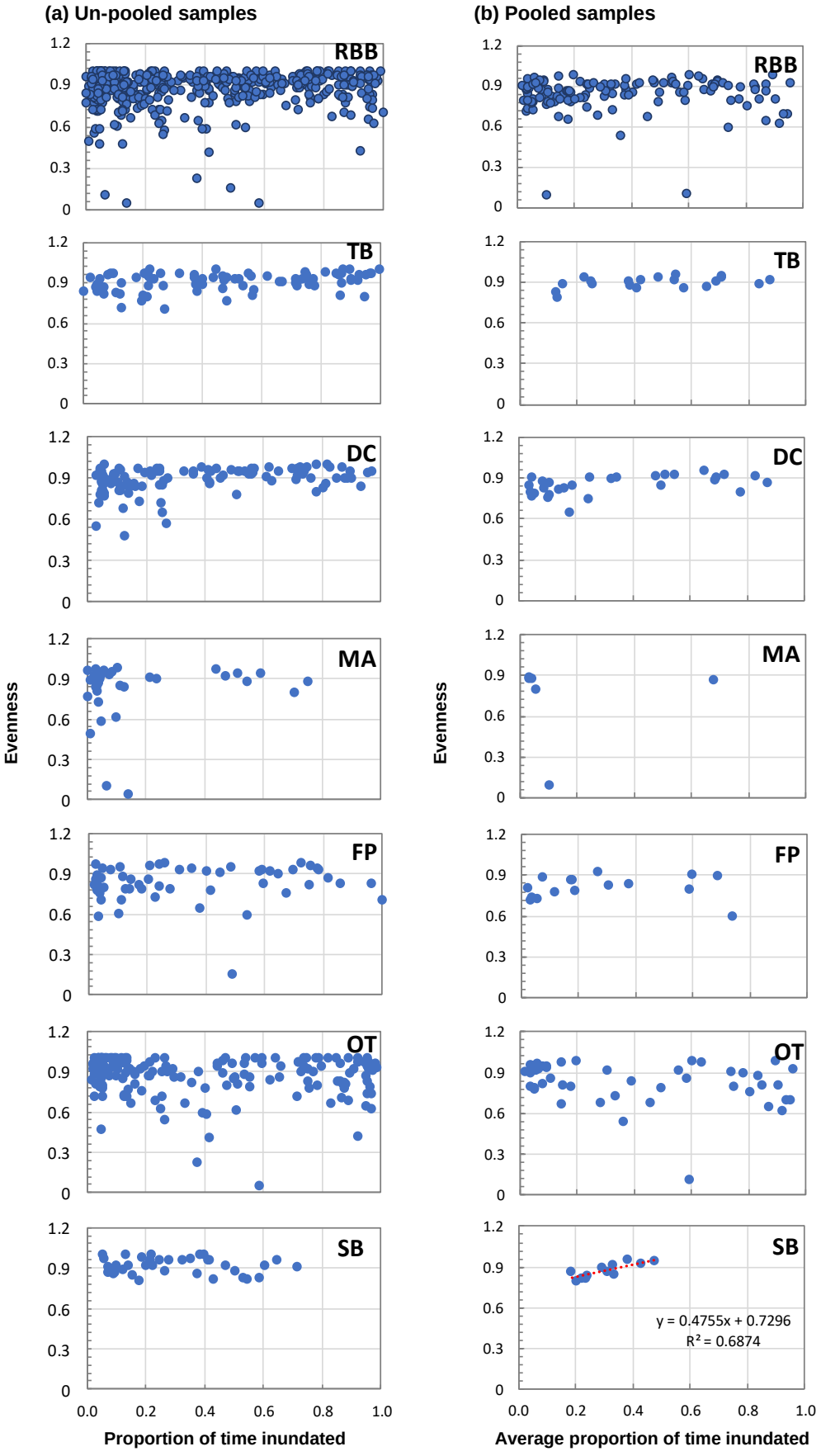


RBB16: Total abundance vs Inundation



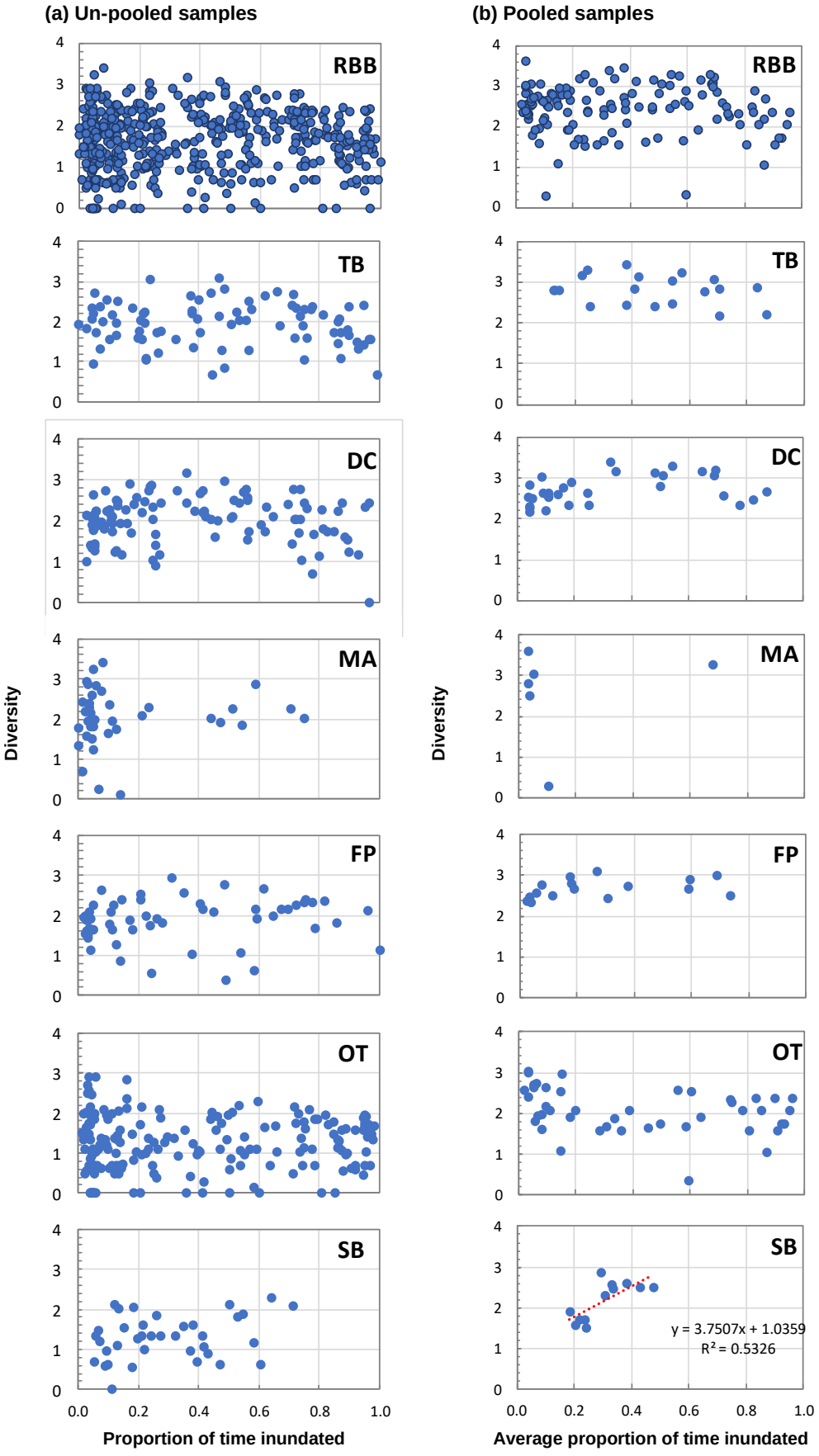
Linear trend line, and regression equation and coefficient are shown for significant ($p < 0.05$) linear relationships

RBB16: Evenness (*J'*) vs Inundation



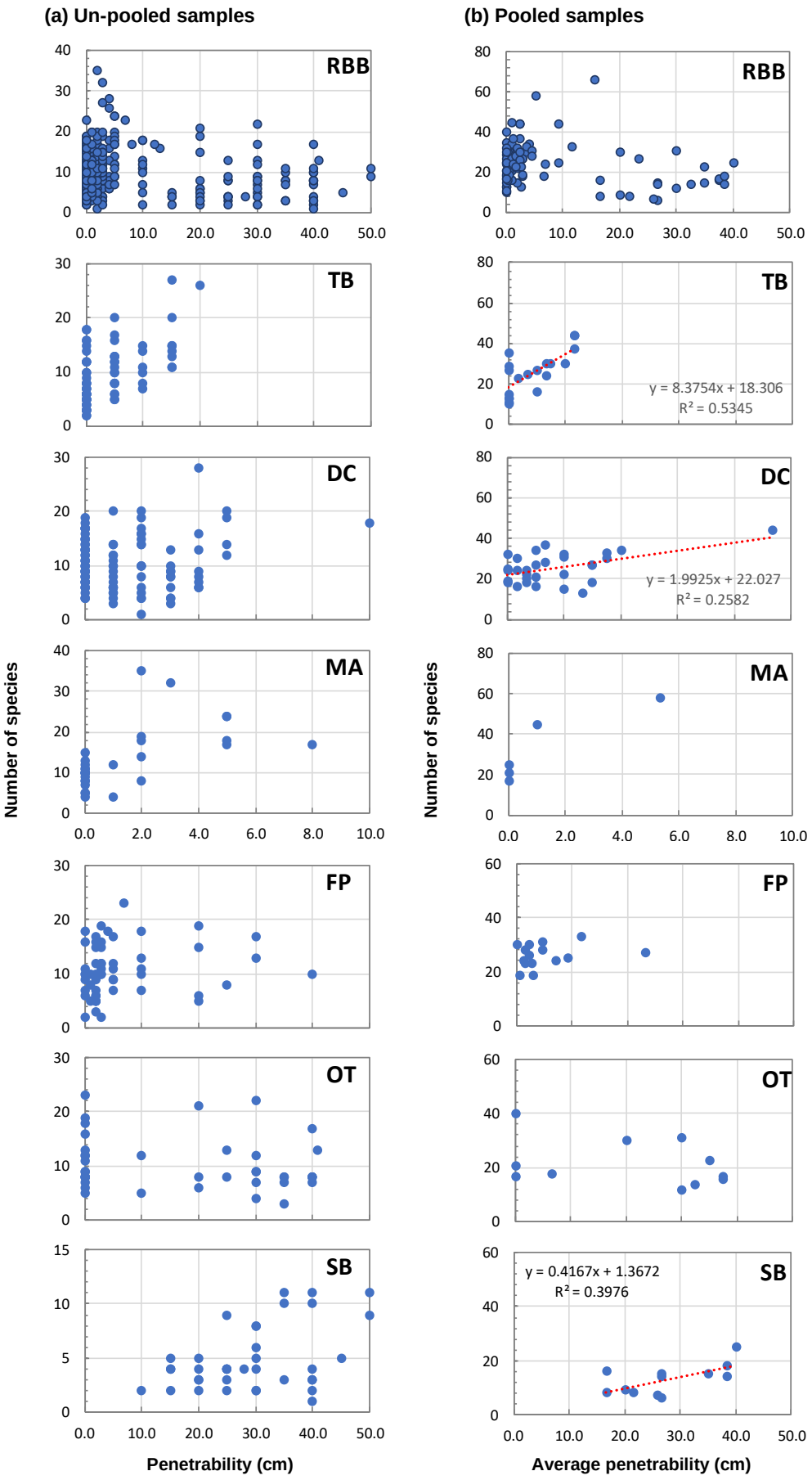
Linear trend line, and regression equation and coefficient are shown for significant ($p < 0.05$) linear relationships

RBB16: Shannon-Weiner Diversity (*H'* $\log_e$) vs Inundation



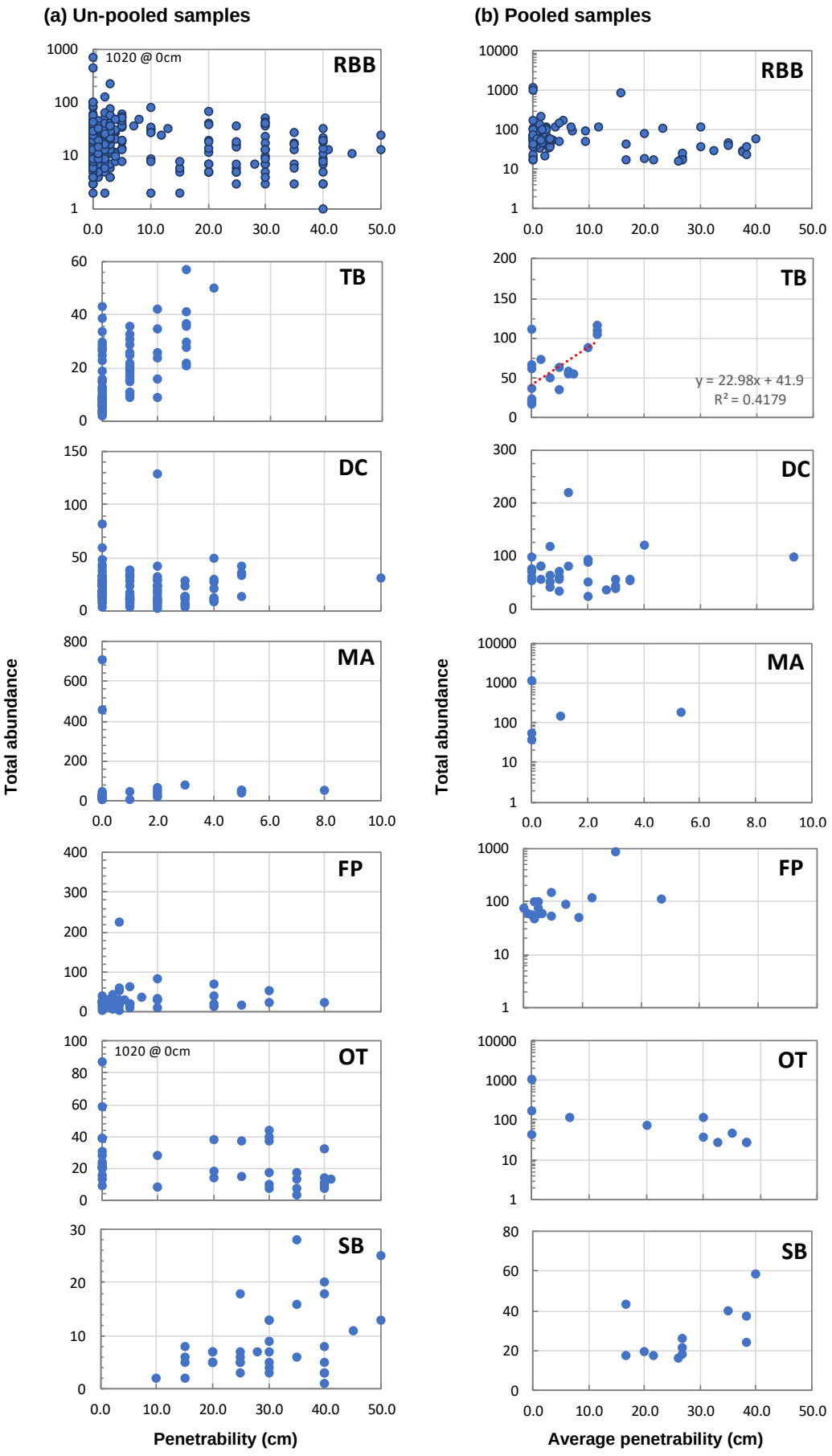
Linear trend line, and regression equation and coefficient are shown for significant ($p < 0.05$) linear relationships

RBB16: Species Richness vs Penetrability



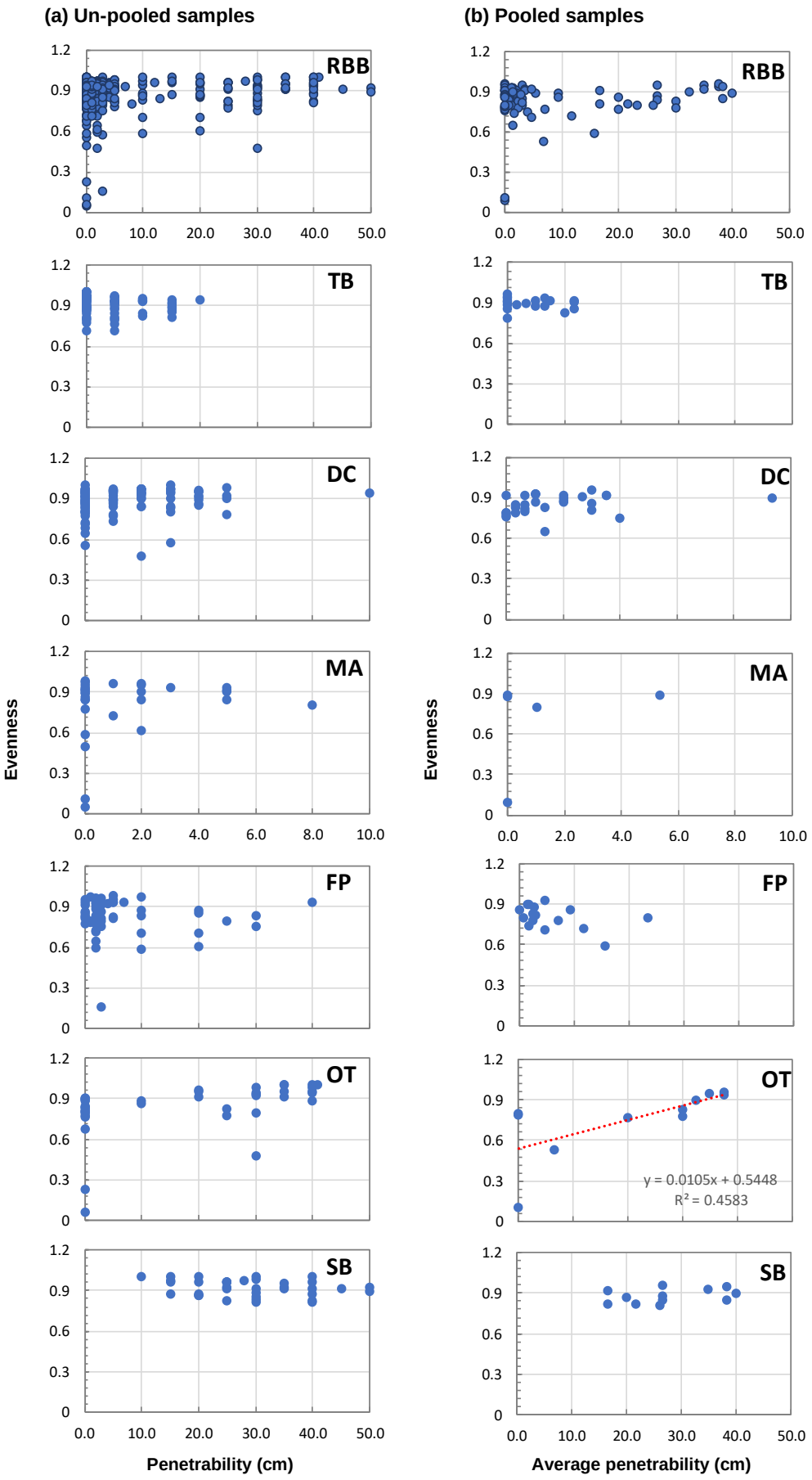
Linear trend line, and regression equation and coefficient are shown for significant ($p < 0.05$) linear relationships. Note, x-axis not the same for all plots.

RBB16: Total Abundance vs Penetrability



Linear trend line, and regression equation and coefficient are shown for significant ($p < 0.05$) linear relationships. Note, x-axis not the same for all plots.

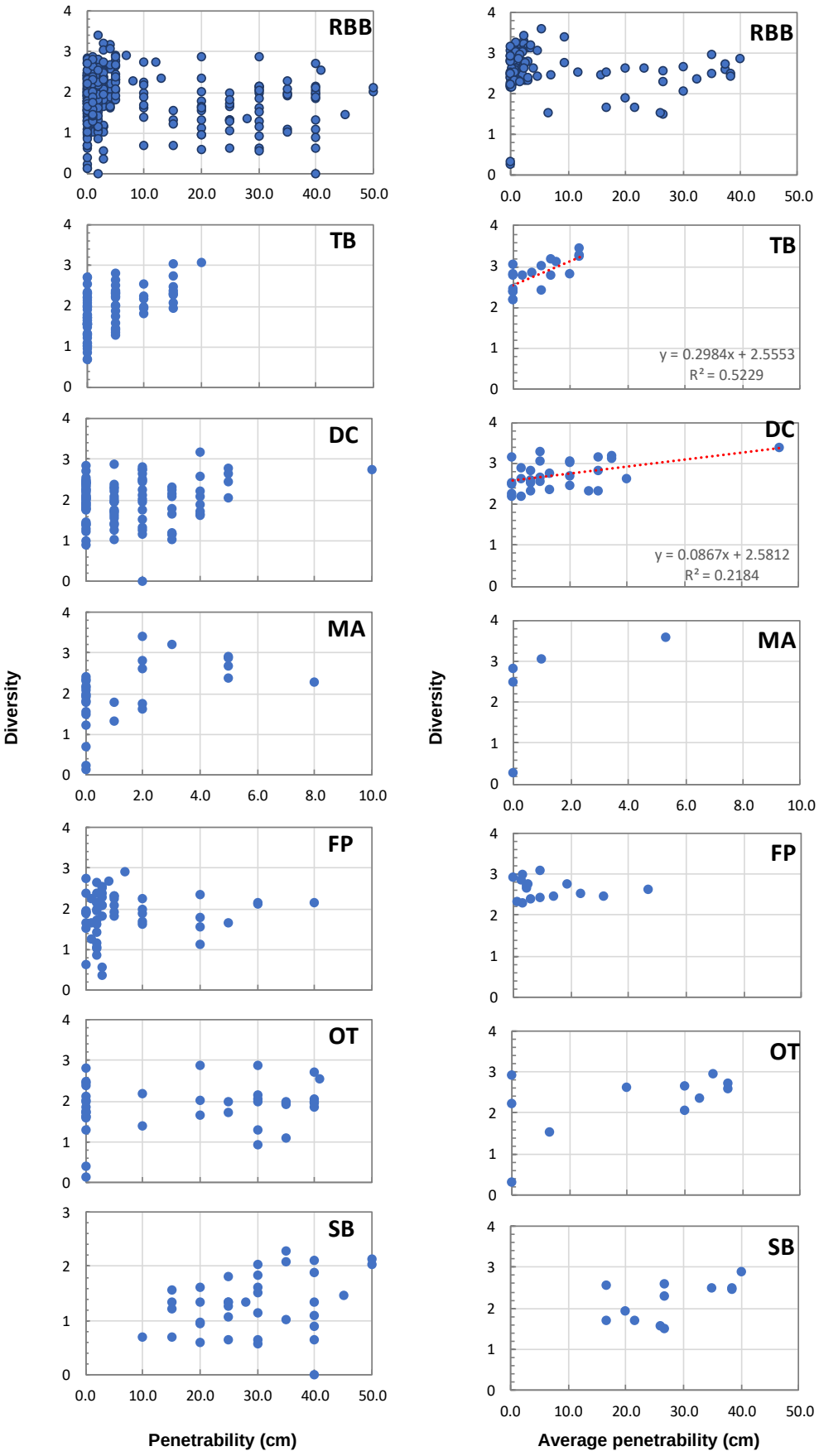
RBB16: Evenness (*J'*) vs Penetrability



Linear trend line, and regression equation and coefficient are shown for significant ($p < 0.05$) linear relationships. Note, x-axis not the same for all plots.

RBB16: Shannon-Weiner Diversity (*H'* $\log_e$) vs Penetrability

(a) Un-pooled samples **(b) Pooled samples**

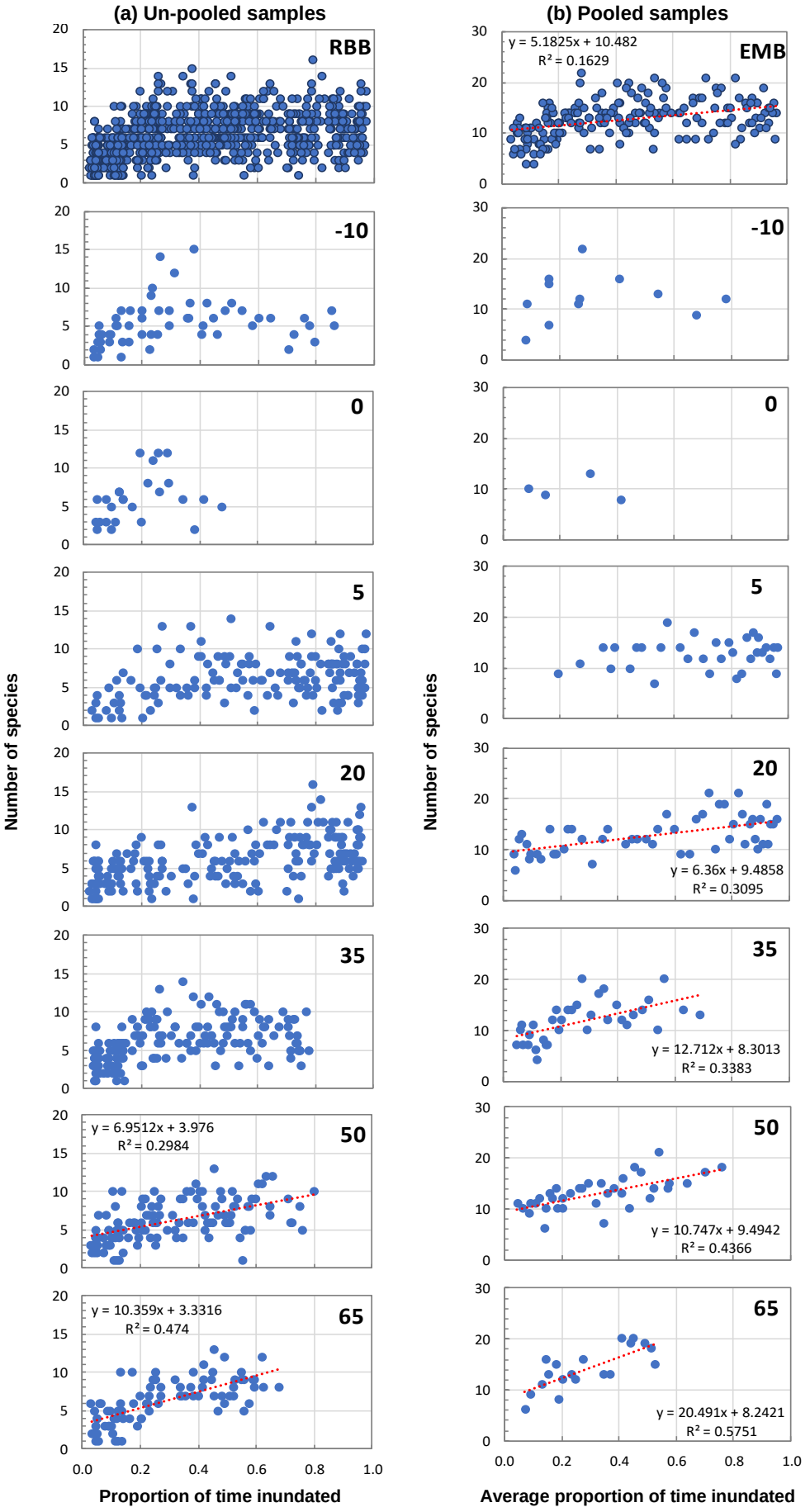


Linear trend line,and regression equation and coefficient are shown for significant ($p < 0.05$) linear relationships. Note, x-axis not the same for all plots.

APPENDIX 2. EMB 2016 SCATTER PLOTS

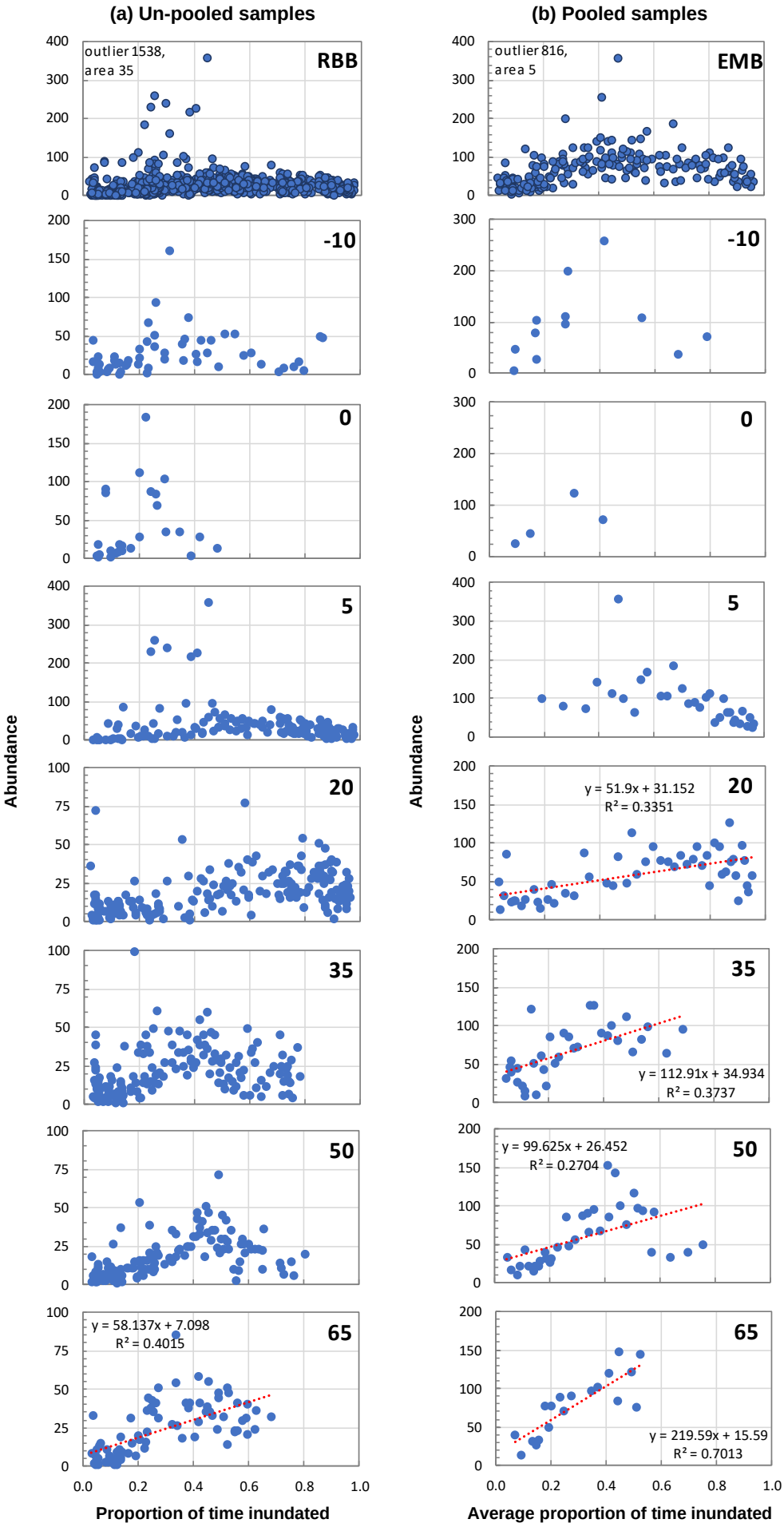
Relationships between species metrics and environmental variables

EMB16: Species Richness vs Inundation



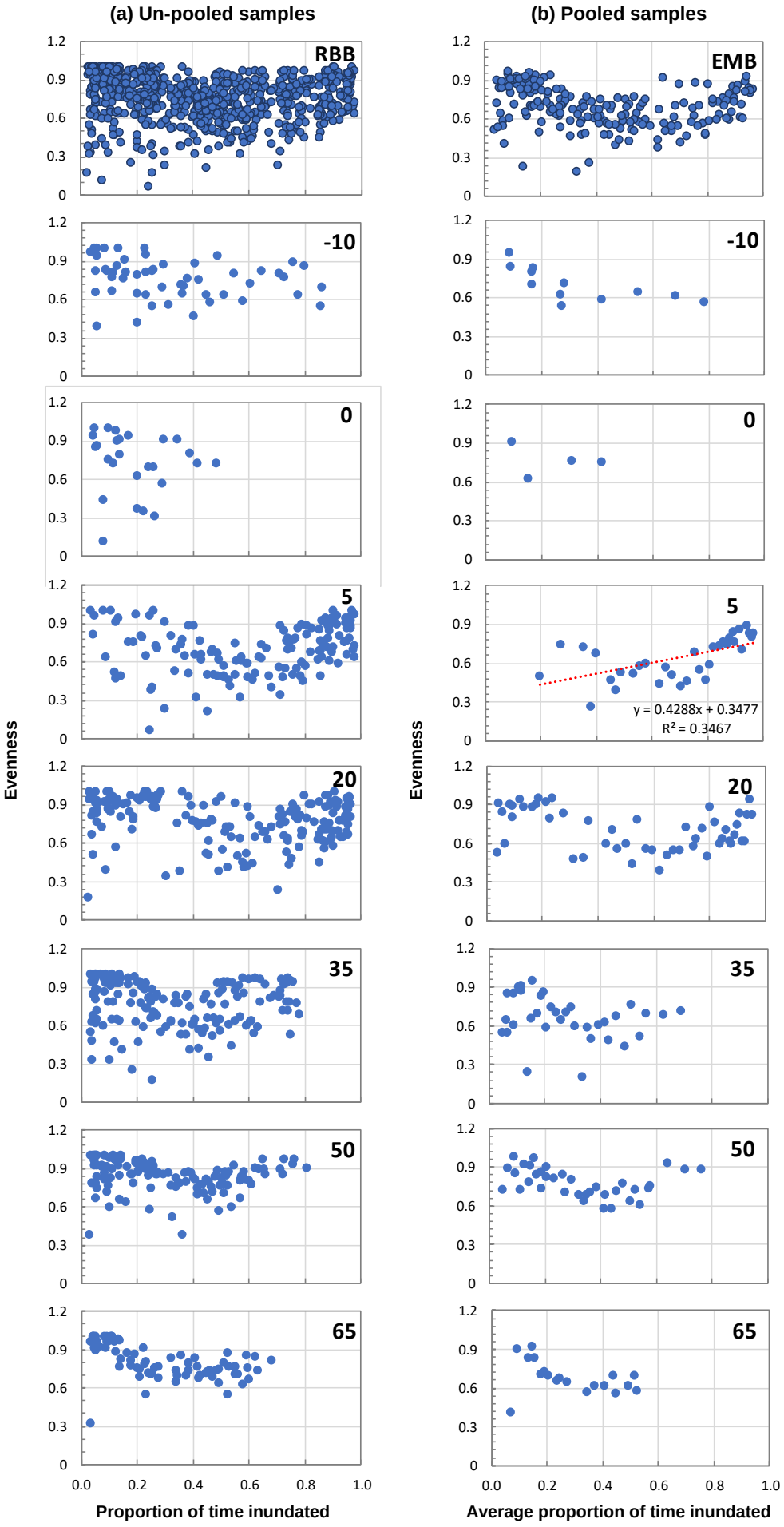
Linear trend line, and regression equation and coefficient are shown for significant linear ($p < 0.05$) relationships

Total Abundance vs Inundation



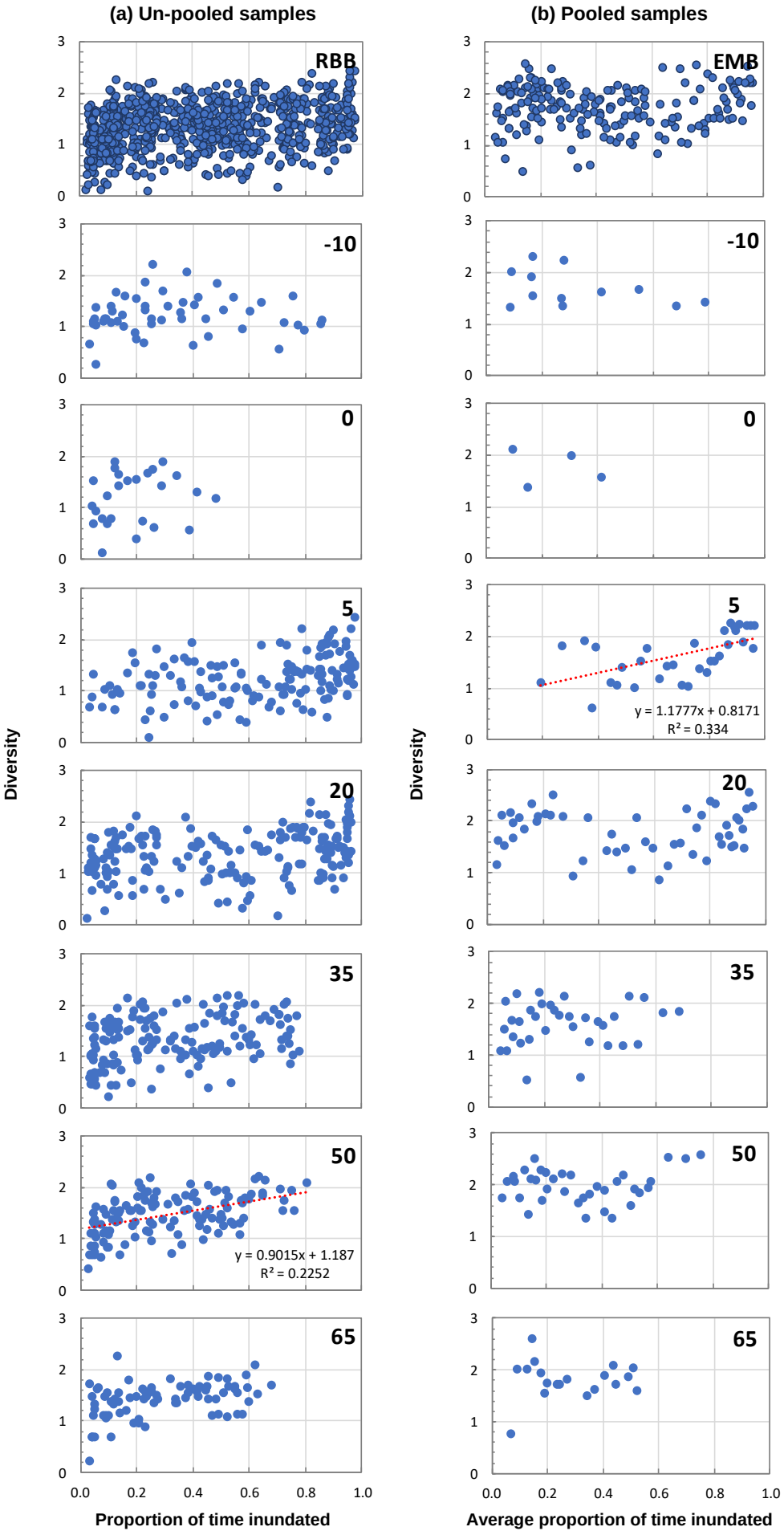
Linear trend line, and regression equation and coefficient are shown for significant ($p < 0.05$) linear relationships

EMB16: Evenness (*J'*) vs Inundation



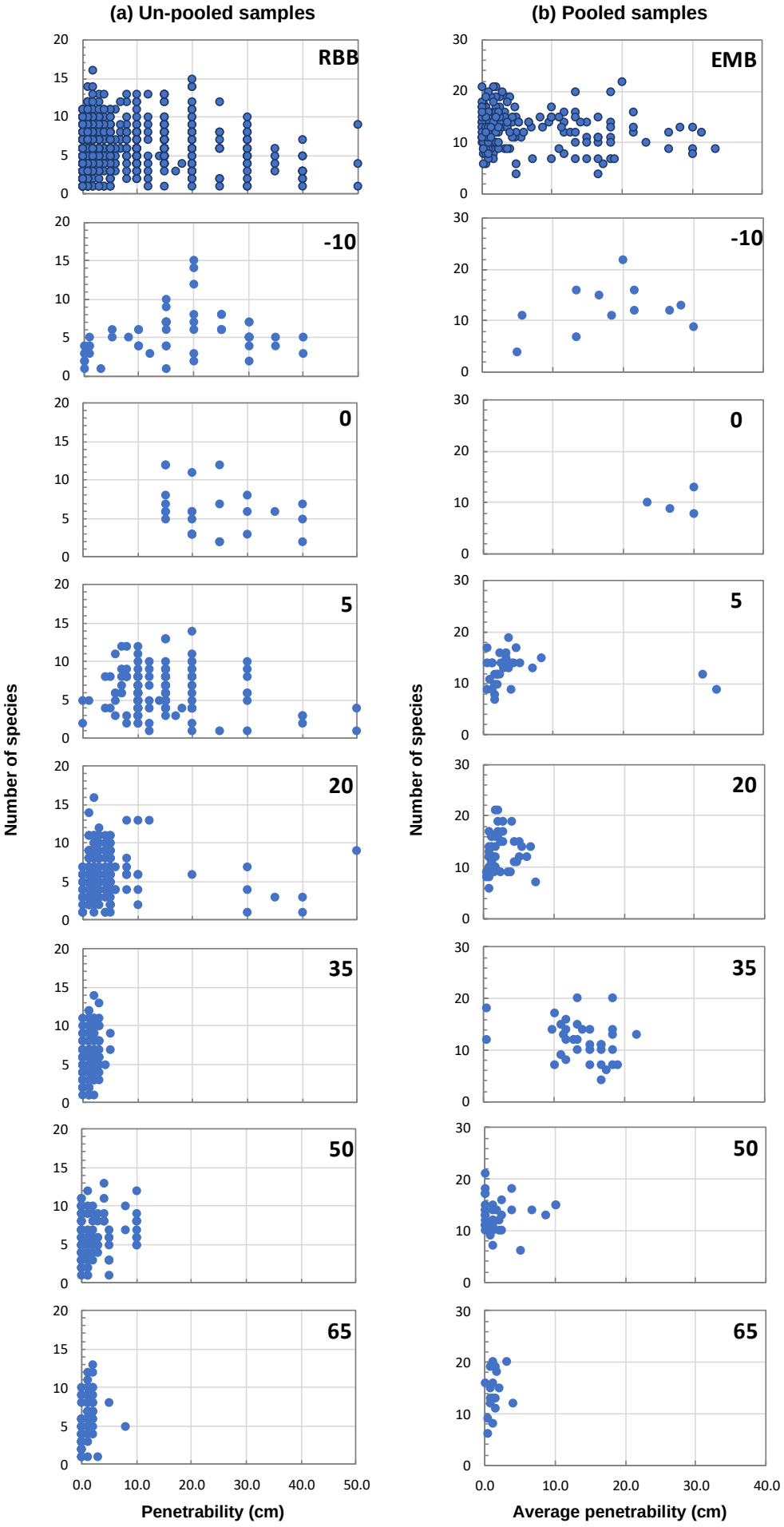
Linear trend line, and regression equation and coefficient are shown for significant ($p < 0.05$) linear relationships

EMB16: Shannon-Weiner Diversity (*H'* $\log_e$) vs Inundation

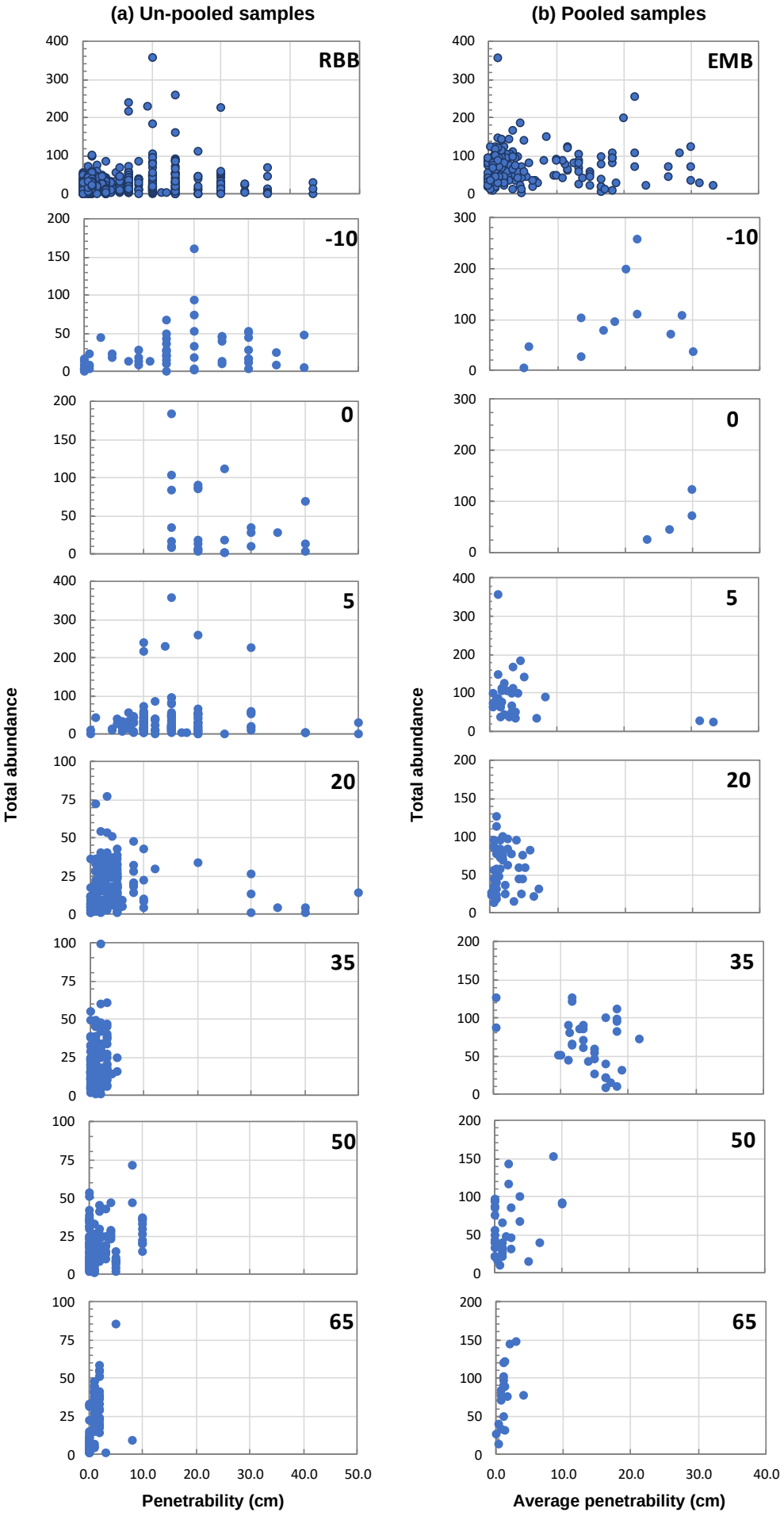


Linear trend line, and regression equation and coefficient are shown for significant ($p < 0.05$) linear relationships.

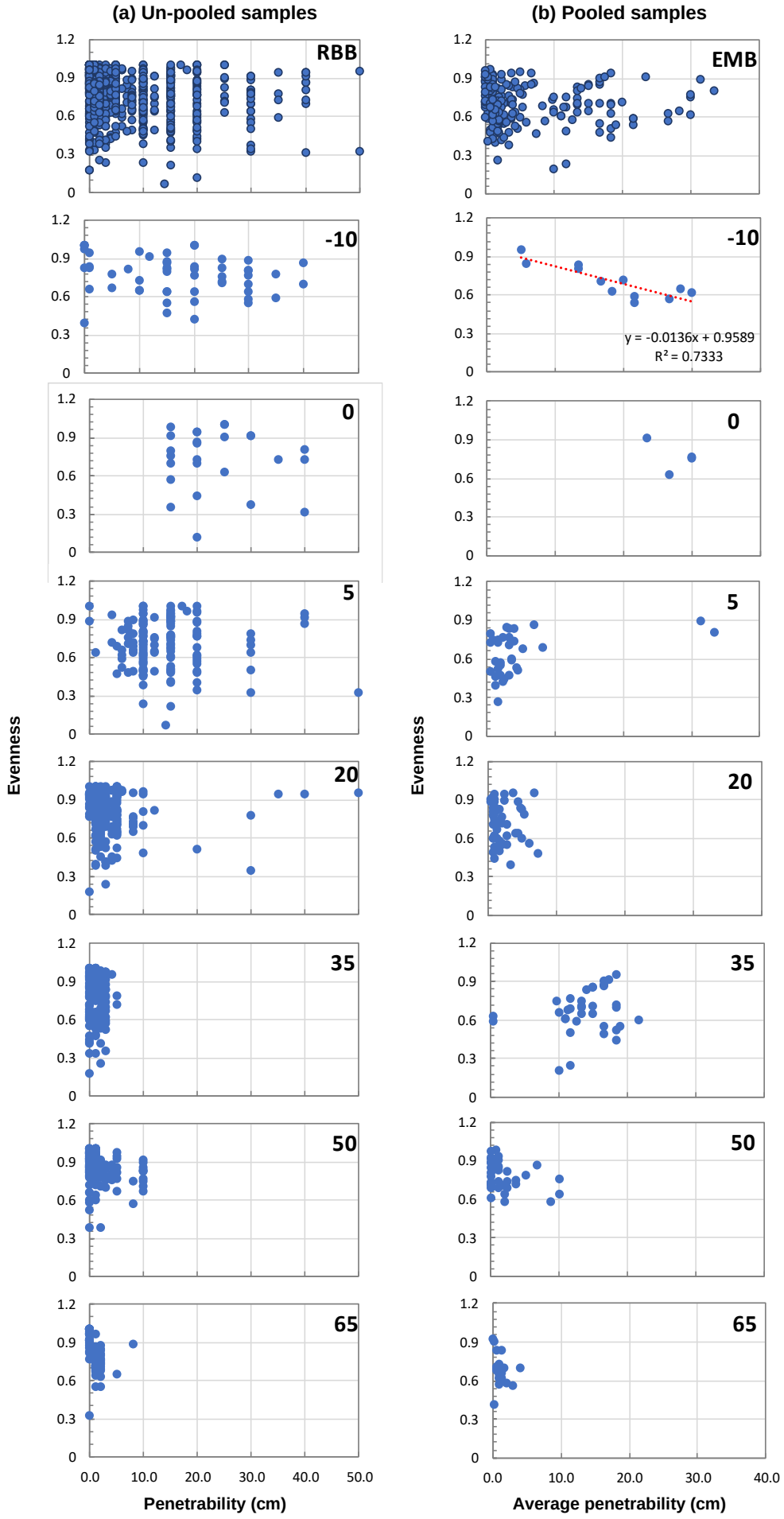
EMB16: Species Richness vs Penetrability



EMB16: Total Species Abundance vs Penetrability

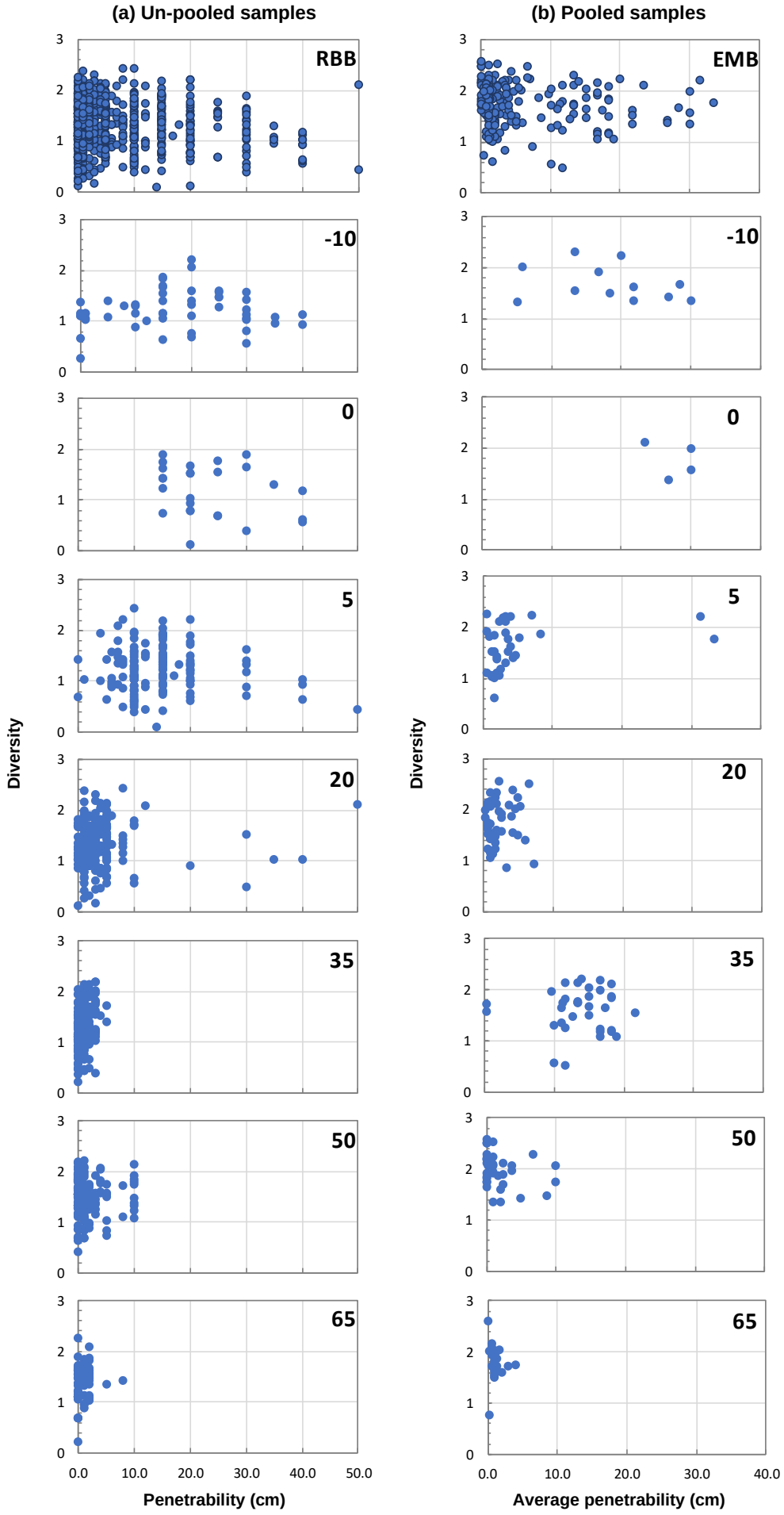


EMB16: Evenness (*J'*) vs Penetrability



Linear trend line, and regression equation and coefficient are shown for significant ($p < 0.05$) linear relationships

EMB16: Shannon-Weiner Diversity (*H'* $\log_e$) vs Penetrability



APPENDIX 3. RB 2016: PERMANOVA TESTING FOR SUB-AREA EFFECTS

Results from PERMANOVA testing for RB sub-area effects on species assemblages (4th root transformed abundance data) within areas (TB, DC, FP, OT SB), including penetrability and inundation categories covariables. Note, there were insufficient samples after pooling to test area MA, and insufficient penetrability data to test for area OT.

RB Area	Source	PERMANOVA (main effects)					
		df	SS	MS	Pseudo-F	Var.	SD
TB	Inundation	1	4395.9	4395.9	2.0898	114.62	10.706
	Penetrability	1	5425.6	5425.6	2.5793	179.13	13.384
	Sub-Area	6	15406	2567.7	1.2207	190.38	13.798
	Inund x Penetr	1	2720.5	2720.5	1.2933	91.831	9.5828
	Inund x Sub-Area	5	10693	2138.6	1.0167	16.692	4.0855
	Penetr x Sub-Area	3	6673.6	2224.5	1.0575	3153.9	56.16
	Inund x Penetr x Sub-Area	0	0		No test	No test	
	Residual	2	4207.1	2103.5		2103.5	45.864
	Total	19	49522				
DC	Inundation	1	18124	18124	9.288	539.09	23.218
	Penetrability	1	3223.8	3223.8	1.6521	49	7
	Sub-Area	4	8294.2	2073.6	1.0626	22.804	4.7754
	Inund x Penetr	1	2534.4	2534.4	1.2988	125	11.18
	Inund x Sub-Area	4	10054	2513.6	1.2881	138.33	11.761
	Penetr x Sub-Area	4	7105.9	1776.5	0.91038	-99.742	-9.9871
	Inund x Penetr x Sub-Area	4	7969.7	1992.4	1.0211	129.4	11.375
	Residual	10	19513	1951.3		1951.3	44.174
	Total	29	76820				
FP	Inundation	1	3895.5	3895.5	2.0678	125.73	11.213
	Penetrability	1	2666	2666	1.4151	59.887	7.7387
	Sub-Area	5	11731	2346.2	1.2454	201.64	14.2
	Inund x Penetr	1	1934.5	1934.5	1.0268	6.1127	2.4724
	Inund x Sub-Area	3	7284.3	2428.1	1.2888	381.49	19.532
	Penetr x Sub-Area	0	0		No test	No test	
	Inund x Penetr x Sub-Area	0	0		No test	No test	
	Residual	4	7535.7	1883.9		1883.9	43.404
	Total	15	35047				
OT	Inundation	1	9051.7	9051.7	3.3059	143.49	11.979
	Sub-Area	4	25904	6476	2.3652	492.32	22.188
	Inund x Sub-Area	4	13875	3468.8	1.2669	171.09	13.08
	Residual	34	93093	2738		2738	52.326
	Total	43	141920				
SB	Inundation	1	4935.7	4935.7	1.8511	189.11	13.752
	Penetrability	1	2143.5	2143.5	0.80391	-67.498	-8.2157
	Sub-Area	2	4223.7	2111.9	0.79204	-411.08	-20.275
	Inund x Penetr	1	2043.1	2043.1	0.76624	-173.95	-13.189
	Inund x Sub-Area	2	4394.9	2197.4	0.82413	-3428.8	-58.556
	Penetr x Sub-Area	2	3474.2	1737.1	0.65149	-3166.6	-56.272
	Inund x Penetr x Sub-Area	1	2554.2	2554.2	0.95793	-1746.4	-41.79
	Residual	1	2666.4	2666.4		2666.4	51.637
	Total	11	26436				

APPENDIX 4. EBB 2016: ANCOVA TESTING FOR SUB-AREA EFFECTS

EMBB 2016: Results from ANCOVA testing for sub-area effects on species metrics within areas (-10, 5 20, 35, 50, 65), including penetrability and inundation categories covariables. Note, there were insufficient samples after pooling to test area 0.

Species richness

EMB Area	Source	df	MS	F	*p*
-10	Inundation	1	5.581	0.202	0.676
	Penetrability	1	1.722	0.062	0.815
	Sub-area	1	4.517	0.164	0.706
	Inund x Sub-Area	1	16.725	0.606	0.480
	Penetr x Sub-Area	1	25.576	0.927	0.390
	Residual	4	27.588		
	Total	12			
5	Inundation	1	0.073	0.011	0.918
	Penetrability	1	4.423	0.654	0.427
	Sub-area	2	1.848	0.273	0.763
	Inund x Sub-Area	2	5.113	0.756	0.480
	Penetr x Sub-Area	2	4.261	0.630	0.541
	Residual	24	6.761		
	Total	33			
20	Inundation	1	159.253	16.793	0.000
	Penetrability	1	0.006	0.001	0.980
	Sub-area	2	7.161	0.755	0.476
	Inund x Sub-Area	2	3.691	0.389	0.680
	Penetr x Sub-Area	2	7.732	0.815	0.449
	Residual	42	9.483		
	Total	51			
35	Inundation	1	111.091	11.088	0.003
	Penetrability	1	4.171	0.416	0.524
	Sub-area	2	1.782	0.178	0.838
	Inund x Sub-Area	2	31.360	3.130	0.061
	Penetr x Sub-Area	2	13.077	1.305	0.288
	Residual	26	10.019		
	Total	35			
50	Inundation	1	37.461	13.684	0.002
	Penetrability	1	0.716	0.262	0.615
	Sub-area	4	15.898	5.807	0.003
	Inund x Sub-Area	4	18.784	6.861	0.001
	Penetr x Sub-Area	4	3.278	1.197	0.344
	Residual	19	2.738		
	Total	36			
65	Inundation	1	86.124	15.157	0.005
	Penetrability	1	40.652	7.154	0.028
	Sub-area	2	1.715	0.302	0.747
	Inund x Sub-Area	2	7.079	1.246	0.338
	Penetr x Sub-Area	2	18.851	3.318	0.089
	Residual	8	5.682		
	Total	19			

Species Abundance (log10 transformed)

EMB Area	Source	df	MS	F	*p*
-10	Inundation	1	0.069	0.319	0.603
	Penetrability	1	0.010	0.045	0.842
	Sub-area	1	0.086	0.397	0.563
	Inund x Sub-Area	1	0.159	0.729	0.441
	Penetr x Sub-Area	1	0.216	0.993	0.375

	Residual	4	0.217		
	Total	12			
5	Inundation	1	1.340	56.665	0.000
	Penetrability	1	0.026	1.115	0.302
	Sub-area	2	0.218	9.236	0.001
	Inund x Sub-Area	2	0.374	15.802	0.000
	Penetr x Sub-Area	2	0.017	0.737	0.489
	Residual	24	0.024		
	Total	33			
20	Inundation	1	0.848	23.888	0.000
	Penetrability	1	0.000	0.008	0.928
	Sub-area	2	0.013	0.361	0.699
	Inund x Sub-Area	2	0.005	0.144	0.866
	Penetr x Sub-Area	2	0.022	0.615	0.545
	Residual	42	0.035		
	Total	51			
35	Inundation	1	1.365	13.601	0.001
	Penetrability	1	0.120	1.200	0.283
	Sub-area	2	0.011	0.107	0.899
	Inund x Sub-Area	2	0.268	2.672	0.088
	Penetr x Sub-Area	2	0.094	0.939	0.404
	Residual	26	0.100		
	Total	35			
50	Inundation	1	0.087	4.209	0.054
	Penetrability	1	0.001	0.059	0.811
	Sub-area	4	0.008	0.383	0.818
	Inund x Sub-Area	4	0.020	0.961	0.451
	Penetr x Sub-Area	4	0.039	1.897	0.152
	Residual	19	0.021		
	Total	36			
65	Inundation	1	0.121	2.614	0.145
	Penetrability	1	0.039	0.831	0.389
	Sub-area	2	0.000	0.005	0.995
	Inund x Sub-Area	2	0.004	0.076	0.927
	Penetr x Sub-Area	2	0.006	0.132	0.878
	Residual	8	0.046		
	Total	19			

Species Evenness (*J'*) (log_{10} transformed)

EMB Area	Source	df	MS	F	*p*
-10	Inundation	1	0.006	1.338	0.312
	Penetrability	1	0.000	0.080	0.792
	Sub-area	1	0.000	0.053	0.828
	Inund x Sub-Area	1	0.005	1.120	0.350
	Penetr x Sub-Area	1	0.004	0.863	0.405
	Residual	4	0.004		
	Total	12			
5	Inundation	1	0.253	18.704	0.000
	Penetrability	1	0.029	2.138	0.157
	Sub-area	2	0.028	2.099	0.144
	Inund x Sub-Area	2	0.075	5.529	0.011
	Penetr x Sub-Area	2	0.002	0.137	0.873
	Residual	24	0.014		
	Total	33			
20	Inundation	1	0.130	5.273	0.027
	Penetrability	1	0.000	0.001	0.976
	Sub-area	2	0.001	0.059	0.943
	Inund x Sub-Area	2	0.013	0.546	0.584
	Penetr x Sub-Area	2	0.001	0.043	0.958

	Residual	42	0.025		
	Total	51			
35	Inundation	1	0.106	4.691	0.040
	Penetrability	1	0.015	0.649	0.428
	Sub-area	2	0.018	0.818	0.452
	Inund x Sub-Area	2	0.019	0.825	0.449
	Penetr x Sub-Area	2	0.003	0.116	0.891
	Residual	26	0.023		
	Total	35			
50	Inundation	1	0.000	0.008	0.932
	Penetrability	1	0.004	0.481	0.496
	Sub-area	4	0.004	0.520	0.722
	Inund x Sub-Area	4	0.007	0.939	0.463
	Penetr x Sub-Area	4	0.010	1.274	0.315
	Residual	19	0.008		
	Total	36			
65	Inundation	1	0.016	1.092	0.327
	Penetrability	1	0.034	2.286	0.169
	Sub-area	2	0.031	2.053	0.191
	Inund x Sub-Area	2	0.042	2.798	0.120
	Penetr x Sub-Area	2	0.016	1.068	0.388
	Residual	8	0.015		
	Total	19			

Species Diversity (Shannon-Weiner)

EMB Area	Source	df	MS	F	*p*
-10	Inundation	1	0.002	0.020	0.893
	Penetrability	1	0.007	0.059	0.820
	Sub-area	1	0.122	1.015	0.371
	Inund x Sub-Area	1	0.035	0.286	0.621
	Penetr x Sub-Area	1	0.092	0.762	0.432
	Residual	4	0.121		
	Total	12			
5	Inundation	1	1.655	14.152	0.001
	Penetrability	1	0.145	1.238	0.277
	Sub-area	2	0.108	0.921	0.412
	Inund x Sub-Area	2	0.371	3.173	0.060
	Penetr x Sub-Area	2	0.012	0.104	0.901
	Residual	24	0.117		
	Total	33			
20	Inundation	1	0.016	0.086	0.771
	Penetrability	1	0.023	0.121	0.729
	Sub-area	2	0.055	0.293	0.747
	Inund x Sub-Area	2	0.108	0.570	0.570
	Penetr x Sub-Area	2	0.110	0.584	0.562
	Residual	42	0.189		
	Total	51			
35	Inundation	1	0.003	0.020	0.889
	Penetrability	1	0.007	0.039	0.845
	Sub-area	2	0.162	0.927	0.409
	Inund x Sub-Area	2	0.076	0.433	0.653
	Penetr x Sub-Area	2	0.060	0.345	0.711
	Residual	26	0.175		
	Total	35			
50	Inundation	1	0.143	1.954	0.178
	Penetrability	1	0.047	0.640	0.434
	Sub-area	4	0.081	1.104	0.383
	Inund x Sub-Area	4	0.111	1.517	0.237
	Penetr x Sub-Area	4	0.061	0.833	0.521
	Residual	19	0.073		

	Total	36			
65	Inundation	1	0.619	5.193	0.052
	Penetrability	1	0.728	6.102	0.039
	Sub-area	2	0.223	1.872	0.215
	Inund x Sub-Area	2	0.452	3.793	0.069
	Penetr x Sub-Area	2	0.371	3.110	0.100
	Residual	8	0.119		
	Total	19			

APPENDIX 5. EMB 2016: PERMANOVA TESTING FOR SUB-AREA EFFECTS

EMB 2016: Results from PERMANOVA testing for sub-area effects on species assemblages (4th root transformed abundance data) within areas (-10, 5, 20, 35, 50, 65), including penetrability and inundation categories covariables.

EMB Area	Source	df	SS	MS	Pseudo F	*p*	Var.	SD
-10	Inundation	1	2418.1	2418.1	1.7557	0.178	86.739	9.3134
	Penetrability	1	3572.4	3572.4	2.5938	0.046	355.56	18.856
	Sub-Area	2	5235.5	2617.7	1.9007	0.077	522.07	22.849
	Inund x Penetr	0	0		No test		No test	
	Inund x Sub-Area	0	0		No test		No test	
	Penetr x Sub-Area	2	3934.8	1967.4	1.4285	0.192	428.61	20.703
	Inund x Penetr x Sub-Area	0	0		No test		No test	
	Residual	5	6886.3	1377.3			1377.3	37.112
	Total	11	22047					
5	Inundation	1	4168	4168	3.395	0.002	89.101	9.4393
	Penetrability	1	2298.3	2298.3	1.8721	0.071	32.443	5.6959
	Sub-Area	2	2498.4	1249.2	1.0175	0.437	2.293	1.5143
	Inund x Penetr	1	1353	1353	1.102	0.357	8.9386	2.9898
	Inund x Sub-Area	2	3114.5	1557.3	1.2685	0.206	42.701	6.5346
	Penetr x Sub-Area	1	1744.3	1744.3	1.4208	0.169	58.657	7.6588
	Inund x Penetr x Sub-Area	0	0		No test		No test	
	Residual	24	29464	1227.7			1227.7	35.038
	Total	32	44641					
20	Inundation	1	18129	18129	13.31	0.001	328.77	18.132
	Penetrability	1	2276.5	2276.5	1.6714	0.059	18.252	4.2722
	Sub-Area	2	4154.3	2077.2	1.525	0.06	45.129	6.7178
	Inund x Penetr	1	1534.2	1534.2	1.1264	0.312	7.0625	2.6575
	Inund x Sub-Area	2	3884.7	1942.4	1.4261	0.076	38.531	6.2073
	Penetr x Sub-Area	0	0		No test		No test	
	Inund x Penetr x Sub-Area	0	0		No test		No test	
	Residual	43	58568	1362.1			1362.1	36.906
	Total	50	88547					
35	Inundation	1	12526	12526	8.605	0.001	316.3	17.785
	Sub-Area	2	4478.5	2239.3	1.5383	0.066	70.17	8.3767
	Inund x Sub-Area	2	3547.8	1773.9	1.2186	0.256	30.258	5.5007
	Residual	29	42215	1455.7			1455.7	38.153
	Total	34	62767					
50	Inundation	1	9851.3	9851.3	7.2896	0.001	236.11	15.366
	Penetrability	1	700.05	700.05	0.51801	0.883	-18.249	-4.2718
	Sub-Area	5	10581	2116.2	1.5659	0.02	145.62	12.067
	Inund x Penetr	1	842.55	842.55	0.62346	0.788	-15.247	-3.9048
	Inund x Sub-Area	3	3720.9	1240.3	0.91778	0.593	-26.467	-5.1446
	Penetr x Sub-Area	3	4940	1646.7	1.2185	0.219	49.559	7.0398
	Inund x Penetr x Sub-Area	1	769.52	769.52	0.56942	0.87	-98.085	-9.9038
	Residual	20	27028	1351.4			1351.4	36.762
	Total	35	58434					
60	Inundation	1	5230.4	5230.4	3.3305	0.003	192.63	13.879
	Penetrability	1	1137.1	1137.1	0.72406	0.647	-23.768	-4.8753
	Sub-Area	3	4667.9	1556	0.99078	0.491	-3.6647	-1.9143
	Inund x Penetr	0	0		No test		No test	
	Inund x Sub-Area	1	824.51	824.51	0.52501	0.877	-113.65	-10.661
	Penetr x Sub-Area	0	0		No test		No test	
	Inund x Penetr x Sub-Area	0	0		No test		No test	
	Residual	12	18846	1570.5			1570.5	39.629

Total		18	30705