Title: An assessment of the seascape genetic structure and hydrodynamic connectivity for sub-tropical seagrass restoration

Running title: Seascape genetics for seagrass restoration

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Abstract

Seagrass ecosystems have suffered significant declines globally and focus is shifting to restoration efforts. A key component to successful restoration is an understanding of the genetic factors potentially influencing restoration success. This includes understanding levels of connectivity between restoration locations and neighbouring seagrass populations that promote natural recovery (source and sink populations), the identification of potential donor populations and assessment of genetic diversity of restored meadows and material used for restoration. In this study we carry out genetic surveys of 352 individuals from 13 populations using 11 polymorphic microsatellite loci to inform seagrass restoration activities by: i) understanding levels of genetic and genotypic diversity within meadows; and ii) understanding genetic structure and patterns of connectivity among these

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meadows to determine which source sites may be most appropriate to assist recovery of three restoration sites. The study identified high genotypic diversity within the locations analysed from the Port of Gladstone and Rodd's Bay region, indicating sexual reproduction is important in maintaining populations. Overall, we detected significant genetic structuring among sites with the Bayesian structure analysis identifying genetic clusters that largely conformed to a northern, central and southern region. This suggests limited gene flow between regions, although there does appear to be some connectivity within regions. The hydrodynamic models showed that seeds were largely locally retained, while fragments were more widely dispersed. Limited gene flow between regions suggests donor material for restoration should be sourced locally where possible.

Keywords: *Zostera muelleri*, connectivity, genetics, dispersal, hydrodynamics.

Conceptual Implications

- Understanding the seascape genetics and connectivity between seagrass meadows is particularly important for seagrass restoration efforts in tropical coasts, since opportunistic seagrass species dominate, and disturbance modifiers result in a higher proportion of transitory seagrass meadow forms (seagrass meadows which are not persistent over time).
- Studying genetic structure and levels of relatedness among populations in association with hydrodynamic models of dispersion can provide evidence of isolation and potential for natural recruitment of seagrass meadows in highly dynamic tropical seagrass seascapes.
- Differences in consistency in the relationships between genetic differentiation versus geographic connectivity, provided insight on other processes relevant to restoration, such as unsuitable conditions for certain genotypes, or the frequency and extent of propagule supply.

Introduction

Over the last 150 years, accelerating rates of coastal urbanization, industrialization, and land reclamation have caused the loss of almost one-third of the world's seagrass habitats (Waycott et al. 2009) impacting critical seagrass ecosystem services, such as nutrient cycling, sediment stabilization, carbon sequestration, and nursery and feeding habitats (Nordlund et al. 2017). The naturally slow rate of seagrass recovery is outpaced by rapid coastal development, encouraging human intervention through restoration, creation, rehabilitation and enhancement. Unfortunately less than 40% of seagrass restoration efforts are deemed a success (Bayraktarov et al. 2016), and few projects have managed to "*restore populations to a level that will allow them to persist over the long term within a dynamic landscape and include the ability to undergo adaptive evolutionary change*" (i.e. the restoration goal of a population biologist, sensu Montalvo et al. 1997). Seagrass restoration literature (mostly around failures) is also northern hemisphere centric (Katwijk et al. 2016).

The recovery of seagrass populations depends on both habitat quality (e.g. water clarity, hydrodynamics, nutrients, sediment dynamics) and the genetic potential of the populations (genetic variation, population structure, adaptation and connectivity) (Ferber et al., 2008). Assessments of habitat quality are common in the management of seagrass populations but genetic surveys to document the genetic seascape are a key step which is often skipped (but see Tanaka et al. 2011). To date, restoration has commonly focused on transplanting seagrass into degraded areas from healthy 'donor' meadows (Tan et al. 2020). Where adult transplants or seeds are taken from a relatively small area there is an increased chance that genotypic and genetic diversity will be low and limit future adaptive potential of restored meadows (Reynolds et al. 2011).

Seascape genetics is the study of how environmental spatial variability influences spatial patterns in the genetics of marine organisms (Riginos & Liggins 2013) and its application to seagrass restoration is being increasingly recognised, with studies showing a lack of genetic diversity, outbreeding and adaption mismatch are impacting the success rate of seagrass restoration projects (Reusch 2005; Jahnke et al. 2015). Genetic and genotypic diversity have long been recognised to convey fitness, stability, resilience and the provision of ecosystem services (Reusch et al. 2005; Hughes & Stachowicz 2011; Connolly et al. 2018) and there is experimental evidence showing the benefits of genetic diversity for enhancing the capacity of seagrass populations to resist stressors such as disease and physical disturbance (Massa et al. 2013). However, the suitability of any translocations needs to be carefully considered as the introduction of generalist or highly competitive genotypes can also result in "genetic swamping" or result in outbreeding depression, which may reduce effective population size and decrease fitness and population resilience (Williams et al. 2014). Nevertheless, restoration efforts typically should look to target local sourced restoration material that is genetically and genotypically diverse to maximise the potential resilience of restored meadows (Sinclair et al. 2014).

Connectivity, between local populations of seagrass may strengthen regional seagrass population (meta-population) resilience by maintaining genetic exchange and allowing re-colonisation of disturbed patches (Bell 2006; Reynolds et al. 2013). Understanding the connectivity of different seagrass meadows provides an important knowledge base for answering questions about why individual seagrass meadows may be declining or recovering and where restoration may make the biggest contribution to wider meta-population survival (Akçakaya et al. 2007). Additionally, the identification of more genetically isolated populations within the wider meta-population may highlight populations that are vulnerable, but also may have acquired distinct evolutionary

adaptations through isolation and selection to local conditions (Connolly et al. 2018). The use of such individuals may result in outbreeding depression if used as donors for locations where environmental conditions differ (Reynolds et al. 2013). Studying genetic structure and levels of relatedness among populations in association with hydrodynamic models of dispersion can provide evidence of isolation and potential for natural recruitment (Reynolds et al. 2013). Best practice for seagrass restoration now recognises the importance of understanding and applying genetic principles to facilitate meadow persistence and expansion (Kettenring et al. 2014).

As Queensland's largest multi-commodity port, the Port of Gladstone supports several major industries. The Port lies within the Great Barrier Reef World Heritage Area, and the environmental management of port operations is highly valued and regulated by state and federal governments. Past declines in the seagrass have been attributed to port development, flood events and poor catchment management, and a direct loss from land reclamation of intertidal areas for capital dredge material disposal (Bryant et al. 2014). Three target restoration locations (Bushy Island, Hummock Hill Island and Wiggins Island, Figure 1) were examined within the context of the seascape genetics. Target locations were selected based on sites where seagrass had been lost or had declined in the last 10 years, but habitat was still suitable. We used a seascape genetics approach to: i) identify how connected the three restoration locations are to 13 surrounding seagrass meadows; ii) characterise the genetic neighbourhood to identify connectivity with potential donor locations; iii) determine if potential donor locations have high genetic and genotypic diversity; and finally iv) find which locations within the seascape are the most vulnerable to future loss because of isolation and genetic differentiation.

Methods

Study location

There are over 100 distinct seagrass meadows in Port of Gladstone and Rodd's Bay in Central Queensland (23° 55' 38" S, 151° 25' 24" E) (Davies et al. 2013) which vary in terms of patch configuration, environment (depth, wave and current exposure, turbidity) and human pressures. The most widely distributed and persistent intertidal species is *Zostera muelleri* subsp. *capricornii* Irmisch ex Ascherson, 1867 (referred to as *Z. muelleri*), (Figure 1). *Zostera muelleri* is found around southern and eastern Australia, New Zealand and Papua New Guinea (Green & Short 2003). In southern and eastern Australia, it has a broad but disjunct distribution related to the availability of suitable habitat (e.g. shallow, sheltered, gently sloping), but reflective of the high dispersal ability of propagules (seeds, spathes and rhizome fragments) and their long viability (Weatherall et al. 2016). Flowering shoots of *Z. muelleri* may remain on the plant releasing seeds locally. In the Port of Gladstone region *Z. muelleri* shoots are relatively short (<20 cm) and flowers (spathes) tend to be within 5-10 cm from the seabed, limiting dispersal of the negatively buoyant seeds when they are released from the attached spathe. Spathes and vegetative fragments may also detach from the plant due to physical disturbance and/or aging and evidence indicates that spathes and vegetative fragments may remain buoyant for a number of days (21 days, Weatherall et al., 2016). McMahon et al. (2014) describe buoyant flowering branches of *Zostera* species being transported up to 100kms over weeks to months.

Sample collection

Collection of genetic material was carried out in September and October 2013. We pre-selected 20 locations across Rodds Bay and the Port of Gladstone based on 2009 maps of location of *Z. muelleri*. Location selection was stratified so that more locations were sampled where seagrass meadow area

was greater. Out of the 20 pre-selected locations, *Z. muelleri* could not be located at seven, leaving 13 meadows available for genetic analysis (see Figure 1). At each meadow a 50 x 50m grid was located over an area of continuous (or as close to) seagrass, and at each of 36 sites (six rows of six sites separated by 10m) one ramet of *Z. muelleri* (at least three shoots long) was collected. Samples were washed of mud and epiphytes removed, before drying in a desiccator for 48 hours.

DNA extraction and genotyping

Genomic DNA was isolated from leaf tissue using DNeasy plant kits (QIAGEN) following the manufacturer's instructions. All samples were genotyped using eleven polymorphic microsatellite markers previous developed for this species; NSWZos02, NSWZos15, NSWZos17, NSWZos18, NSWZos19, NSWZos20, NSWZos23, NSWZos25, NSWZos29, NSWZos38 and NSWZos46 (Sherman et al. 2012). Microsatellites were amplified using a polymerase chain reaction (PCR) conducted in 11 µL volumes containing; 10 ng of genomic DNA; 5 µL PCR Master Mix (Qiagen, USA) and 4 µL primer multiplex (0.26 µM of each forward primer and fluorescent dye, 0.13 µM of reverse primer). Thermal cycling conditions for the PCR were; initial hot start at 94°C for 15 min; ten cycles of 94°C for 45 s, 60°C for 45 s, 72°C for 45 s; ten cycles of 94°C for 45 s, 57°C for 45 s, 72°C for 45 s; 20 cycles of 94°C for 45 s, 55°C for 45 s, 72°C for 45 s; final elongation at 72°C for 15 min. PCR amplicons were electrophoresed using an ABI 3130xl Genetic Analyzer, incorporating LIZ 500 (-250) size standard (Applied Biosystems). Alleles were scored using GeneMapper, v3.7 (Applied Biosystems).

Statistical analyses

To assess the independence of loci for genetic analysis we tested each pair-wise combination for linkage disequilibrium within each location using GENEPOP on the web (Rousset 2008). As asexual

reproduction can generate non-random associations between loci and mimic physical linkage, tests for linkage were carried out using only unique multi-locus genotypes (MLG). We found no evidence of linkage between any loci across locations from a total of 715 possible pairwise tests after the application of a sequential Bonferroni correction (Rice 1989). Each locus was tested for the presence of potential null alleles within each site using MICROCHECKER (Van Oosterhout et al. 2004). Potential null alleles were detected at four loci; NSWZos19 within 4 locations (North Pelican Bank – Mid (NPM), North Pelican Bank – South (NPS), Facing Island (FI) & South Trees (ST), see Figure 1), NSWZos38 within South Pelican Bank – South (SPS), NSWZos46 at Grahams Creek (GC), Facing Island and South Trees and NSWZos17 at South Trees. There were however no consistent patterns of null alleles across loci and locations and therefore no data were excluded from analyses.

Estimates of genotypic diversity and the detection of multi-locus genotypes

The relative importance of sexual versus asexual reproduction within a population was determined by first comparing the number of unique MLG detected to the number of samples collected within each site (*N*). MLG can occur through resampling of the same clone (asexual reproduction) or the recombination of the same alleles in different individuals during sexual reproduction. The probability of identical MLG arising from different sexual reproductive events was assessed using P_{SEX} using the statistical software Genclone (Arnaud-Haond & Belkhir 2007). P_{SEX} values ranged between <0.001 and 0.019, suggesting a low probability that the same MLG would have arisen from separate sexual events. Thus, individuals with an identical MLG were assumed to be clones. Genotypic diversity (clonal richness *R* = MLG-1/N-1) and clonal heterogeneity (Simpson's diversity index D*) were calculated using the program Genclone (Arnaud-Haond & Belkhir 2007). Only complete genotypes were used in the assessment of genotypic diversity and any samples where loci failed to amplify

were removed from the analysis. Genotypes shared between different geographical locations may result from the dispersal and recruitment of asexual propagules, or may represent separate sexual events resulting in the same genotype and this was assessed in Genclone (P_{SEX}).

Population structure and genetic diversity

A key assumption underlying many population structure analyses is conformation of loci to Hardy-Weinberg expectations (HWE). Deviations from HWE were tested for each locus using GENEPOP Version 4.2 (Rousset 2008). The Markov chain method was used to estimate exact P-values for each combination using the default settings. To reduce the chance of type I errors, we applied a sequential Bonferroni correction (Rice 1989). Of 122 single locus tests across the 11 loci, we detected 21 significant departures from HWE, however, only one locus (NSW23) showed consistent departures from HWE across 11 of the 13 locations. This locus was removed from all subsequent analyses. Patterns of genetic diversity across locations were expressed as the mean number of alleles per site (Na), observed heterozygosity (H_O) and expected heterozygosity (Nei's (1978) unbiased estimate H_E) using the statistical program Arlequin (Excoffier & Lischer 2010). As clonal reproduction may influence estimates of population structure, all analyses were calculated using unique MLG only. Levels of genetic differentiation were assessed using the following analyses. Firstly, population genetic structure was examined using standardized F-statistics (Weir & Cockerham 1984) in Fstat (Goudet 1995). Secondly, the relationship between geographic and genetic distance was determined by comparing the association between matrices of pairwise F_{ST} / (1 - F_{ST}) and the shortest geographical distance (at high tide) connecting locations by sea. The significance of any patterns were assessed using a MANTEL test (Sokal 1979) using 10 000 permutations with the web based software Isolation by Distance (Jensen et al. 2005). Thirdly, network analysis was done to

visualise genetic relationships between locations and is represented by locations (nodes) and number of edges between nodes (degree). Network analysis was based on F_{ST} estimates and the percolation threshold set to the lowest value that includes all nodes, which was 0.05 in this analysis. Network analysis was done using Edenetworks (Kivelä et al. 2014). The size and colour of each node represents the number of shortest paths that pass though that node (betweenness centrality) and estimates the importance of each site in maintaining gene flow (Kivelä et al. 2014). The width and colour of each edge represents the number of connections between those locations. A Bayesian analysis was used to further visualise genetic structuring and determine the number of genetic clusters within our data set using the program STRUCTURE 2.3.2 (Pritchard et al. 2000). Population clustering was tested at levels K = 1 – 13, with a burn in length of 100,000, and with 100,000 iterations at each level and 10 replicates for each K. Structuring was tested using the admixture model with allelic frequencies correlated among populations and ignoring prior population information (Hubisz et al. 2009). From the STRUCTURE output, the true number of clusters (K) was determined by looking at the optimum ΔK (Evanno et al. 2005) using STRUCTURE HARVESTER (Earl & vonHoldt 2012)(see Figures S1 and S2).

Hydrodynamic connectivity

In order to identify potentially valuable propagule source locations, specific particle tracking was carried out for *Z. muelleri* fragments and spathes combined, and seeds. Vegetative fragments were used as a proxy for spathe dispersal in this analysis, given their similarity in size and shape and similar settling properties. The numerical modelling was performed using the sediment transport module within the flexible mesh finite volume solver TUFLOW-FV (www.tuflow.com). Fragments and spathes were simulated to be buoyant for 21 days before adopting a still water settling velocity of

2E-04 (m/s) and seeds were buoyant for 0 days with a still water settling velocity of 1E-02 (m/s) (Weatherall et al. 2016). 2.52E07 kg of propagules were released over a 7-hour window spanning the high tide within the spring tides (21/01/2012 06:30 to 21/01/2012 13:30) and from within a 250m radius of the release points (see Figure 1). The mass released in the modelling is orders of magnitude higher than reality, to satisfy mass limits within the TUFLOW-FV engine and results provide the relative, rather than the absolute, contribution or connectivity of different meadow locations. The conditions selected for the 31-day tracking simulation were representative of predominant wind conditions during peak seed production and included two spring and two neap tidal cycles under different conditions. Following the simulation, the mass of propagules was extracted from within a 250 m radius of the release points. To appreciate the sensitivity to the prevailing conditions, the mass of propagules was first extracted during a spring tide and quiescent wave conditions and again during a neap tide when winds were generating appreciable waves within the harbour. Simulating the model under different tide and wave resulted in no major changes in the representation of connectivity presented here (Figure S3). The data is presented as matrices of the final mass (divided by 1000 for display) of particles deposited at each location from each release point. Dispersal potential was estimated for both seeds and fragments/spathes by calculating the maximum mass of propagules dispersing between each pairwise combination of locations. Prior to analysis the multiplicative inverse mass of propagules was calculated to reflect that more dispersal was indicative of closer "distance".

Results

Levels of Genotypic Diversity and the detection of multi-locus genotypes

A total of 352 *Z. muelleri* samples from 13 locations were genotyped at 11 loci. Overall, we detected high levels of genotypic diversity within our samples with a total of 293 unique MLG detected across all 13 locations. A total of 17 replicate genotypes were identified, however, most were represented by only two or three individuals; the highest number of clones (5 clones) and greatest number of times a single clone was sampled (7 times) both occurred at the South Trees site. There were 6 locations, Black Swan (BS), North Pelican Bank – South, South Pelican Bank – South, Facing Island, Turkey Beach (TB) and Wiggins Island (WI) where all samples displayed unique multilocus genotypes. Global genotypic diversity was high (R = 0.83) and ranged between R = 0.64 at South Trees and R = 1.0 at locations where all samples had unique MLG (Table 1). As would be expected, high genotypic diversity translates into high clonal heterogeneity (distribution and abundance of clones) with values ranging between 0.94 at South Trees and 1 at locations where only unique MLG were recorded. One MLG was shared between North Pelican Banks – North (NPN) and Grahams Creek and estimates of the probability that these may have resulted from separate sexual events was relatively low (P_{SEX} = 0.0002) and thus may have resulted from the dispersal and recruitment of clonal fragments from the same plant.

Population structure and genetic diversity

Overall, the mean number of alleles per locus across all locations was 3.51 ± 0.62 (SE), and ranged from 2.55 ± 0.34 (SE) at Grahams Creek to 4.09 ± 0.83 (SE) at PS (Table 1). Expected heterozygosity across all locations was 0.43 ± 0.08 (SE), varying between 0.49 ± 0.08 (SE) at Black Swan and 0.38 ± 0.07 (SE) at Grahams Creek. Observed heterozygosity were consistently higher than expected heterozygosity. Overall observed heterozygosity was 0.48 ± 0.01 (SE), and ranged between 0.41 ± 0.09 (SE) at South Pelican Bank – South and 0.57 ± 0.10 (SE) at Black Swan.

Overall, we detected significant genetic structure among locations with a global F_{ST} of 0.104 ± 0.025 (SE) which was significantly different from zero (95 % CI = 0.057 to 0.148). Pairwise estimates of F_{ST} revealed only 12 non-significant values out of a total of 78 pairwise comparisons (Table 2). Nine of the 12 non-significant pairwise comparisons were between the Pelican Bank locations (North Pelican Bank – Mid, North Pelican Banks – North, North Pelican Bank – South and South Pelican Bank – South, Figure 1).

The Bayesian STRUCTURE analysis identified up to four genetic clusters within our data with ΔK largest for K = 4 (mean LnP(K) = -5656.95, ΔK = 31.80; Figure 2). This included a northern cluster (locations Black Swan, Worthington (WO), Grahams Creek and Wiggins Island), a central cluster (locations North Pelican Banks – Mid, North Pelican Banks – North, North Pelican Bank – South, South Pelican Bank – South, and Facing Island), site Bushy Island was identified as a distinct genetic cluster from the central region, and a southern cluster (locations South Trees, Wild Cattle Island (WCI) and TB) (Figure 2).

Network analysis identified 3 main networks which were largely consistent with the northern, central and southern genetic clusters identified in the STRUCTURE analysis (Figure 3). This included a northern cluster with connections among Black Swan, Worthington, and Wiggins Island (but not Grahams Creek). The central network has the strongest connection among sites with the Pelican banks sites well connected to each other. Included in this network were Facing Island and South Trees which appear to be important connecting sites to the southern network (Figure 3). Interestingly, Bushy Island was not connected to this central network and appears largely isolated. The southern network consisted of Wild Cattle Island and Turkey Beach, both of which had

connections to South Trees, suggestion some gene flow between the southern and central networks (Figure 3).

Hydrodynamic connectivity

Matrices of connectivity based on the simulated hydrodynamic dispersal of *Z. muelleri* fragments and spathes (Figure 4a), and seeds (Figure 4b) represent the proportion which came from a particular location (out of the 13 locations examined) assuming equal coverage and biomass at all locations (in order to assess potential connectivity). The diagonal is indicative of propagule retention. Matrices of connectivity of all major seagrass meadows in the Port of Gladstone based on the simulated hydrodynamic dispersal of *Z. muelleri* fragments and seeds, under low wave spring tides (tide dominated) and high wave neap (wave dominated) are shown in Figure S3 for reference. For the vegetative fragments and spathes (Figure 4a) local retention is low, with only Worthington (Figure 1), Graham's Creek and southern Facing Island locations showing any major retention of vegetative fragments and reproductive spathes. Fragment settlement distribution (the number of locations where fragments and spathes settled) varied across locations. Worthington, Graham's Creek and Wild Cattle Island showed the highest local retention of fragments and spathes, and the most deposition from other locations, with most fragments and spathes deposited at one site from another seen at Wild Cattle Island from Turkey Beach. Pelican Banks locations (North Pelican Banks – North, North Pelican Bank – Mid, North Pelican Bank – South, South Pelican Bank – South), Facing Island and Turkey Beach showed little to no deposition of fragments and spathes from other locations within the modelled period.

Local retention of seeds was greater than fragments and spathes, based on the number of locations where seeds did not travel. Wild Cattle Island showed a retention of seeds that was an order of

magnitude greater than other locations, although Black Swan, Worthington and Graham's Creek all showed large seed retention under the modelled conditions. Discounting local retention, Wiggin's Island, Black Swan, Worthington acted as seed sources for the most locations. Wild Cattle Island, Turkey Beach and Northern Pelican Banks North locations were the least important sources for other locations. There was no relationship between genetic differentiation and dispersal potential for seeds ($p = 0.152$, $r^2 = 0.1307$) or fragments and spathes ($p = 0.488$, $r^2 = 0.0016$).

Discussion

An understanding of the genotypic diversity and connectivity is critical to advancing our approaches to seagrass restoration in dynamic seascapes where resilience has emerged as a critical trait for restoration success (York et al. 2017; Statton et al. 2018). The study identified high genotypic diversity within the locations analysed from the Port of Gladstone and Rodd's Bay region, indicating sexual reproduction is important in maintaining populations, which is consistent with previous studies of this species at other locations (Macreadie et al. 2014; Sherman et al. 2016). The results of this study also highlight that even in a well-mixed estuarine system, spatial patterns are observable and provide important insights and approaches which can be used for advising restoration and conservation efforts.

The observed high genotypic diversity has positive implications for restoration. Previous studies have identified that genetic diversity enhances resilience to disturbance in seagrasses (Hughes & Stachowicz 2004; Reusch et al. 2005). Seagrass seeds are of importance in dispersal and recruitment to drive genetic diversity and the use of seeds in restoration has been shown to help maintain genetic diversity in *Zostera marina* restoration during large scale broadcast seeding (Reynolds et al., 2012). Hughes & Stachowicz (2011) conducted experimental biomass removal within seagrass

monocultures and polycultures and found that genotypic diversity enhanced seagrass (*Zostera marina*) resilience, but only at the highest levels of disturbance. They also found that enhancement processes included both trait-independent complementarity (TIC; differential resource use among clones) and positive dominance (due to one genotype achieving high density in both monoculture and polyculture). This has implications for the choice of donor meadows, since selecting high genotypically diverse meadows may improve recovery potential. Selecting from such donor meadows could promote both TIC and positive dominance in the transplants at their new location, increasing the potential for local adaptation to the transplant locations and the maintenance of genetic diversity across the meta-population. However, it is noted that with microsatellites we are unable to comment on adaptive potential of genotypes to local conditions as these are neutral loci and provide no direct information on adaptive potential.

Applying these results to the potential locations for restoration (Bushy Island, North Hummock Hill Island and Wiggins Island), informs decisions on potential for natural recovery, persistence and suitable donor meadows. Wiggins Island close to the mouth of the Calliope estuary has historically had substantial seagrass meadows, which have declined in recent years (Bryant et al. 2014). Unlike Bushy Island, Wiggins Island samples showed strong genetic clustering with the northern locations in The Narrow at Black Swan and Worthington, but also close association with Grahams Creek. Propagule tracking simulations support this, with Wiggins Island receiving propagules primarily from Black Swan and Worthington, but less from Grahams Creek. Propagule supply is low and there is low local retention, which may both limit natural recruitment and recovery at Wiggins Island, and may also make them vulnerable to future loss. Given the genetic similarities between Wiggins Island and Black Swan, Worthington and Grahams Creek, these meadows may act as the most appropriate donor meadows if restoration material needs to be sourced. However, the data suggest that seagrass at Black Swan has

higher genetic and genotypic diversity which may promote greater genetic diversity and resilience at the proposed transplant site (Hughes & Stachowicz 2004).

High genotypic diversity within meadows is likely a result of large effective metapopulation size, frequent intermediate disturbance and some connectivity between some meadow locations (Ferber et al. 2008). Tropical seagrass meadows, such as the ones in Port of Gladstone, are subject to frequent natural disturbance from tropical storms (McKenna et al. 2015), grazing by dugong and turtles, and bioturbation (Vonk et al. 2008). In areas such as the Port of Gladstone where human activity is concentrated they are also impacted by anthropogenic disturbances, including dredging plumes, land reclamation, nutrient enrichment, boat groundings and propeller scarring and, for some intertidal regions, vehicle damage (York et al. 2015). Physical disturbance can enhance local clonal diversity both by creating gaps which are then colonised by seeds, but also by alleviating the monopolisation of space by competitively superior genotypes (Reusch 2006). There is also correlation under some circumstances for greater levels of sexual reproduction of seagrass (*Zostera marina*) under stress or disturbance (Cabaco & Santos 2012), which may manifest in greater genotypic diversity in areas prone to intermediate disturbance.

Species of *Zostera* are known to show large dispersal potential (from 100s to 1000s of km; Kendrick et al. 2012; Smith et al. 2018) due to the positive buoyancy of both fragments and spathes containing seeds (Weatherall et al. 2016). Modes of dispersal can include biological dispersal (e.g. via herbivory) and sediment movement, but the main external, abiotic, dispersal vector for seagrasses is water movement (McMahon et al. 2014), the complexities of which influence propagule dispersal at different temporal and spatial scales (from tidal fluxes to mesoscale eddies and stochastic storm

events). Even following the settlement of seeds, secondary dispersal of seeds may occur due to tidal flow or wind wave induced bedload movement, resuspension or saltation (McMahon et al. 2014).

Knowledge of connectivity and local retention can also inform restoration. For example, propagule tracking simulations also suggested that connectivity to other meadows of Wild Cattle Island, which is adjacent to a potential restoration site at Hummock Hill, is high, with seeds and fragments sourced from all sampled location (including those geographically distant), but specifically from nearby Turkey Beach. There is also substantial local retention at this site. Assuming the adjacent Hummock Hill is similarly well-connected, natural recolonization may be high. Wild Cattle Island is a sandy location with higher light levels than many of the other locations sampled, especially in the Inner Harbour and The Narrows (Baird et al. 2016), which may not be suitable conditions for some of the genotypes. Genotypically similar meadows to Wild Cattle Island seagrass include South Trees and Turkey Beach, both of which have high genotypic diversity and may be good donor meadows for restoring Hummock Hill meadows. Whilst there is an argument for using donor meadows with high genotypic diversity to enhance resilience and genetic diversity in the transplant locations, Wild Cattle Island may also be a good donor for Hummock Hill due to the similarity in habitat conditions.

At the scale of 10s of km in a highly mixed environment such as an estuary we would expect to find high connectivity between local populations. However, levels of genetic differentiation (F_{ST}) and the Bayesian STRUCTURE analysis suggest limited gene flow between some sites. This is likely driven by the hydrodynamically complex nature of the study area, with varying degrees of connectivity and isolation between different sites. Comparisons of genetic differentiation and dispersal potential (of seeds and fragments) showed no significant relationship. This may result because the models predict dispersal, but not necessarily recruitment. While propagules may be transport readily between many

sites, most of these are unlike to successfully recruit. Of those that do recruit, site specific conditions may select for locally adapted genotypes suited to local conditions, thus limiting gene flow (dispersal and integration into the breeding population) between ecologically similar sites (Connolly et al 2018).

STRUCTURE analysis identified Bushy Island as a distinct genetic cluster from the central region. Pairwise FST suggests that Bushy Island is more genetically similar to North Pelican Banks sites, yet contemporary connectivity from the model shows limited connectivity between the two. It is perhaps not surprising that there is a no significant relationship here since the genetic data represent multiple generations of connectivity relative to the model. Seed tracking data, however, suggested that seeds from all other locations may reach the Bushy Island site, and that there is high local retention. Despite this there is limited seagrass growth at this site. The models indicate limited seed dispersal to Bushy Island from the larger more persistent meadows of seagrass in the harbour (e.g. Pelican Bank, South Trees or Rodds Bay), which may provide a more regular supply of propagules. Another, explanation may be that the conditions at this site are not suitable for some genotypes. In terms of restoration, the data suggest that enhancing the habitat to encourage propagule settlement and survival may be more appropriate than the transplantation of propagules. Enhancing the habitat may lead to natural recolonization from other meadows and once established seagrass may be able to persist at this location due to high propagule retention. Due to high local retention and limited supply of propagules to most other meadows the site may not contribute significantly to the resilience of the wider metapopulation.

The occurrence of one shared MLG between the southern end of Pelican Bank North and Graham's Creek suggests connectivity exists even between geographically separated populations. A moderate

P_{sex} value in this context suggests that this is likely the result of dispersal of asexual fragments. The occurrence of only one MLG suggests very limited establishment of vegetative material between meadows despite plants regularly fragmenting and floating some distance. Fragment tracking indicates that during low wave and high current periods Grahams Creek locations are highly connected (in terms of immigration) to other locations within the Port of Gladstone and even Rodd's Bay. South Trees is a less persistent meadow (Bryant et al. 2014), but these results suggest that it has an important role in the connectivity of northern and southern *Z. muelleri* populations with the wider Port of Gladstone area and Rodds Bay region. Restoration efforts which aim to maximise seagrass persistence at this site may therefore have a large impact.

The lack of consistency in the relationships between genetic differentiation versus water dispersal distances could also be due several potential factors. Firstly, alternative methods of dispersal not captured by the model are contributing to migration (McMahon et al. 2014). Secondly, the dispersal models estimate movement of propagules but do not capture recruitment, survival and interbreeding (required for gene flow to have occurred and to effect genetic structure). So even if two sites are well connected, if the propagules are not suited to the new site and die, then no gene flow has occurred. Combining the genetics and hydrodynamic models provides multiple lines of evidence for theories regarding connectivity and demography, for example the importance of key locations in the connectivity of populations and maintenance of the metapopulation.

Whilst the validation of these proposed approaches for restoration is in actual field restoration trials, this study demonstrates the potential value of gaining an understanding of local genetic structure to marry with the more commonly quantified environmental conditions, to collectively enhance probability of restoration success. The rapid development of molecular techniques, that now utilise

powerful genetic markers that can look at local adaptation, are an obvious next step to enable matching genetically suitable material to local restoration locations. This will allow for the selection of donor material that can build resistance into restored populations so that they are better equipped to survive predicted future environmental change (Williams et al. 2014; Breed et al. 2019).

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List of Tables

Table 1 Summary of genotypic and genetic diversity measures for *Zostera muelleri* at meadows within the Port of Gladstone and adjacent Rodds Bay. N = number of samples, D = Simpson's D, MLG = number of multilocus genotypes, R = clonal richness, NA = mean number of alleles/locus, H_O = observed heterozygosity, H_E = expected heterozygosity, F_{is} = inbreeding coefficient.

Meadow	N	D	MLG	R	NA	H_O	H_E	F_{IS}
Black Swan (BS)	32	1.00	32	1.00	3.73	0.57	0.48	-0.19
Worthington (WO)	23	0.98	20	0.86	3.18	0.48	0.43	-0.12
Grahams Creek (GC)	23	0.97	17	0.73	2.55	0.44	0.38	-0.17
Wiggins Island (WI)	28	1.00	28	1.00	3.91	0.54	0.48	-0.12
North Pelican Bank North (NPN)	18	0.98	16	0.88	3.36	0.46	0.41	-0.12
North Pelican Bank Mid (NPM)	36	0.98	35	0.97	3.73	0.48	0.42	-0.15
North Pelican Bank South (NPS)	36	1.00	36	1.00	3.73	0.52	0.41	-0.27
South Pelican Bank SPS	13	1.00	13	1.00	3.18	0.41	0.41	0.00
Facing Island (FI)	33	1.00	33	1.00	4.09	0.46	0.46	0.01
Bushy Island (BI)	34	0.99	31	0.91	3.73	0.46	0.43	-0.08
South Trees (ST)	34	0.95	22	0.64	3.91	0.45	0.49	0.08
Wild Cattle Island (WCI)	7	0.95	6	0.83	2.64	0.49	0.43	-0.13
Turkey Beach (TB)	35	1.00	35	1.00	3.91	0.43	0.41	-0.05
Total	352		293	0.83	3.51	0.48	0.43	

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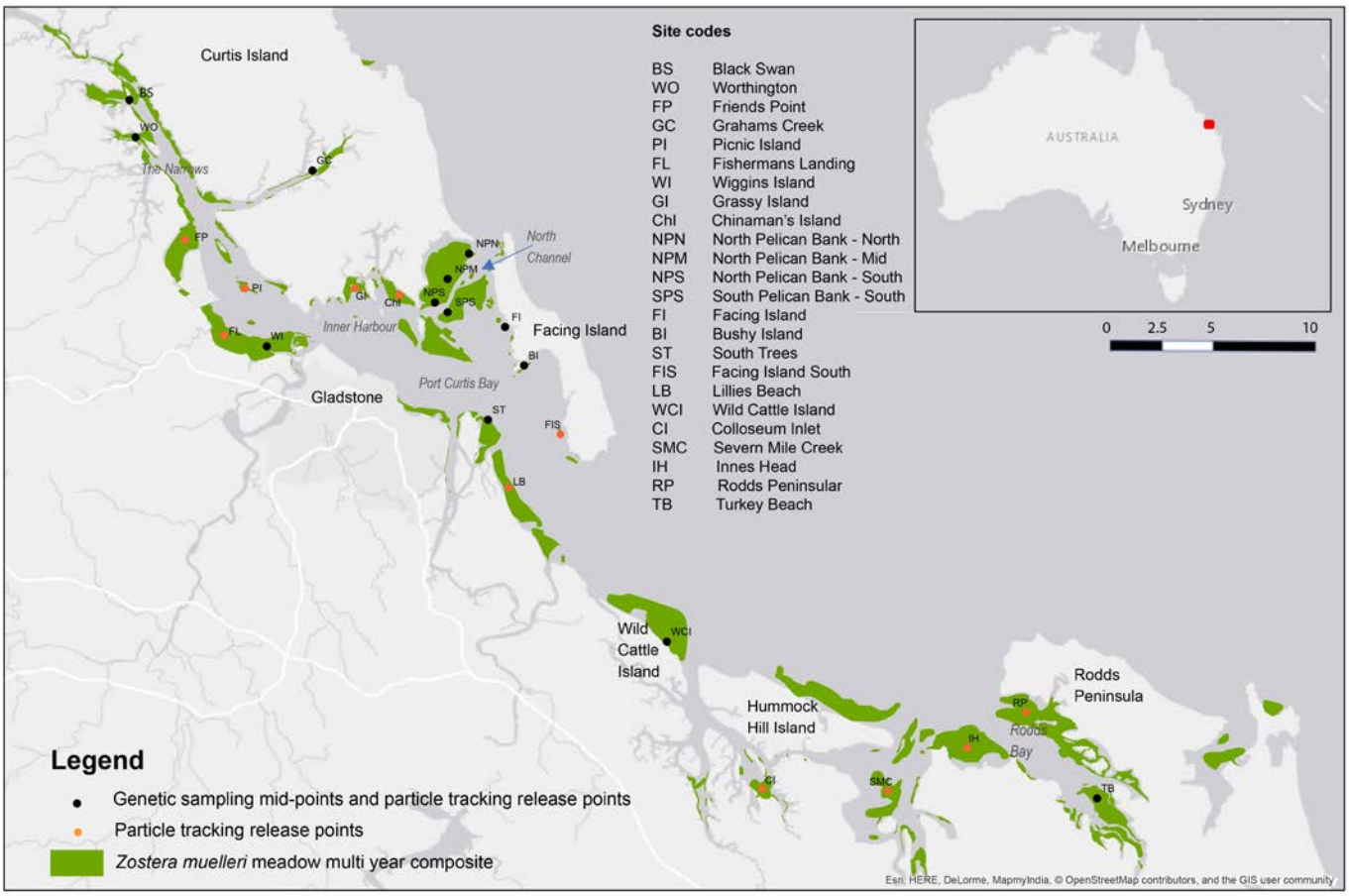

Figure 1

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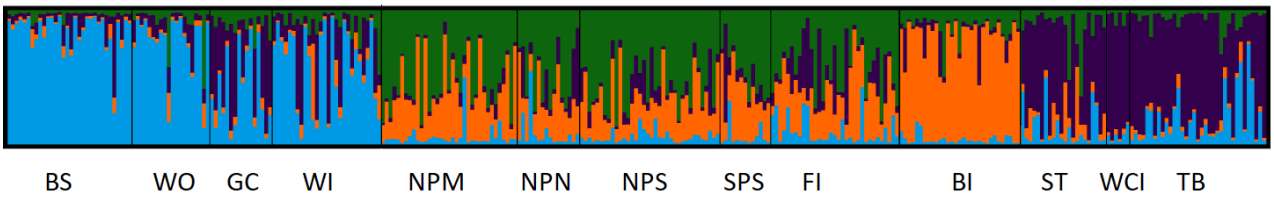

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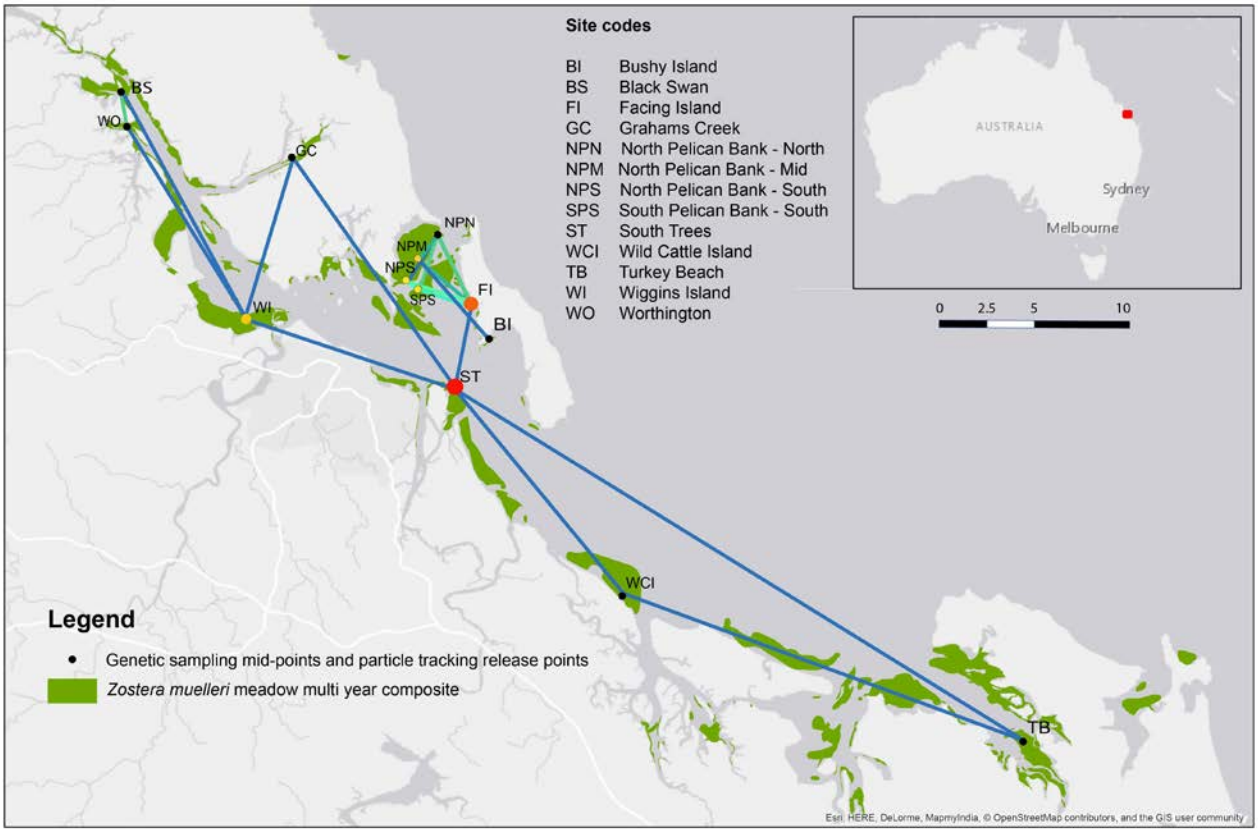

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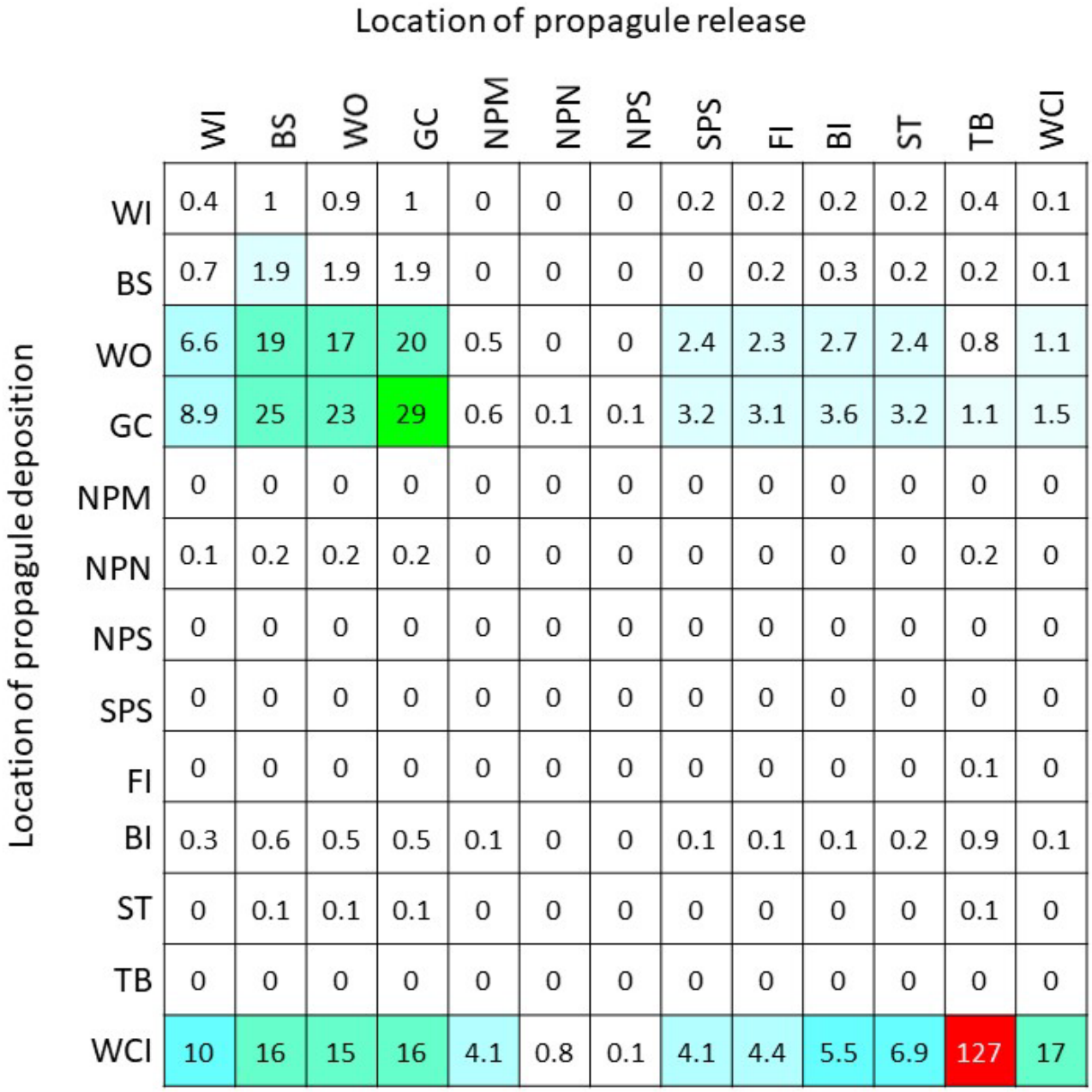

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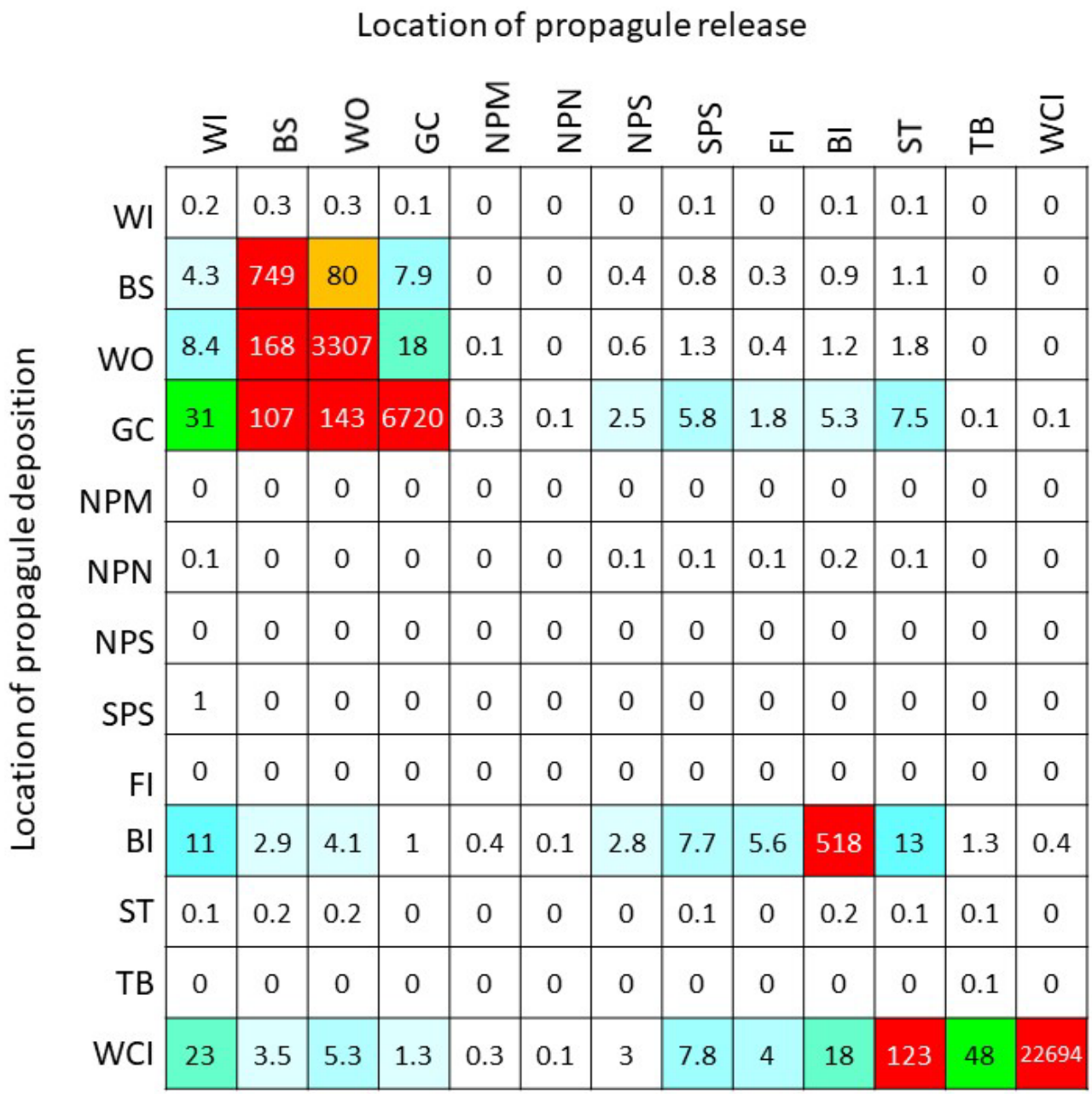

Figure 4b