# Connectivity of the seagrass, *Zostera muelleri,* within south-eastern Australia.

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## Abstract

Contemporary oceanic conditions and local dispersal of propagules influence the genetic diversity and connectivity among seagrass populations. The degree of connectivity between populations of *Zostera muelleri* in south-eastern Australia is unknown. We examined genetic connectivity among 25 sites containing *Z. muelleri* using nine polymorphic microsatellite DNA loci. We hypothesized minimal sharing of genetic material between distant populations and a degree of connectivity between local populations. Genotypic diversity was high with 64% of populations having unique multi locus genotypes (MLG), indicating the importance of sexual reproduction. Two sites shared MLGs, which may be due to the dispersal and recruitment of vegetative propagules. Genetic differentiation was observed between most sites. With the exception of two outlying sites, two genetic population clusters were identified across the studied

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populations. Regionally, the populations have high clonal diversity, are strongly differentiated and generally exist in isolation from one another. Non-significant, within-estuary differentiation, however, was observed for three estuaries indicating a degree of connectivity. The results of this research improve our understanding of the connectivity of *Z. muelleri* populations in the region, an important process for managing this ecosystem engineer.

**Keywords:** Seagrass, microsatellite, connectivity, clonal diversity

## Introduction

Seagrasses are ecologically important, highly specialised angiosperms that provide a multitude of benefits to the systems they inhabit. These benefits include the attenuation of water flows and an increase of sedimentation that provides firm substrata for further colonisation by macroalgae and invertebrates (Bos et al. 2007). Seagrasses also provide substantial nutrient cycling services and nursery habitat for economically important fish and prawn species (Edgar et al. 2001; Walker et al. 1999; Waycott et al. 2009). *Zostera muelleri* is a dominant seagrass found within waters of Australia and New Zealand and has a small distribution within the Torres Strait and Papua New Guinea (ALA 2015). Plants flower during the warmer months and germination of seeds increases under cooler sea surface temperature (15–20 °C) and reduced salinity (<16ppt) (Stafford-Bell et al. 2016; Walker et al. 2001). The species produces large numbers of small (≈2mm) negatively buoyant seeds that are either released directly into the water column or encased within a spathe on positively buoyant reproductive shoots (Ackerman 1997; Ackerman 2006). Vegetative fragments can be dislodged from the sediment through both natural (e.g. wave action,

consumption by large herbivores) or anthropogenic (e.g. propeller scarring, dredging activities) processes. The ability of vegetative fragments to remain both buoyant and viable for extended periods (>5w) indicates a strong dispersal potential for these vegetative tissues (Erftemeijer et al. 2006; Lanyon and Sanson 2006; Stafford-Bell et al. 2015).

The potential re-colonisation of seagrass propagules following periods of dispersal may ensure connectivity between local and regional populations is maintained. This process and the resultant sharing of genetic material between those populations has long been recognised as an important means of maintaining resilience to disturbance as well as facilitating evolutionary processes (McMahon et al. 2018). In fact, where historical barriers to connectivity have existed, such as the Bassian Isthmus, which once connected mainland Australia with Tasmania, clearly defined phylogeographic gaps exist today. Notable examples of this disjunction in the region studied include those between populations of the seagrass, *Posidonia australis* (Sinclair et al. 2016), the pelagic blue blubber jellyfish (*Catostylus mosaicus*) (Dawson 2005), the intertidal gastropod *Nerita* (Waters 2008; Waters et al. 2010) and the common seadragon (*Phyllopteryx taeniolatus*) (Wilson et al. 2017).

Dispersal of propagules and connectivity of *Z. muelleri* populations is dependent upon a range of factors including the reproductive biology of the species, propagule form and ultimately the influence of oceanic and local hydrology (McMahon et al. 2018). However, although connectivity of populations may exist through propagule dispersal, the low success of transplantation studies and natural reattachment does not ensure successful

recruitment (Di Carlo et al. 2005; Thomson et al. 2014). Furthermore, given flowering in seagrasses is limited to a very small proportion of the population (≈ 10%), the low ability of seeds to disperse and high mortality of seedlings (roughly 2% of seedlings will survive past the first year) (Hemminga and Duarte 2000), diversity of populations could be expected to be low. Should recruitment occur, immigrating genetic individuals supplement the genetic diversity within populations leading to an increased resilience of those populations to disturbance (Procaccini et al. 2007; Sherman et al. 2016). Maintenance of genetic diversity and supplementation of populations from surrounding sources therefore allows the persistence of a group of populations within a given area (a metapopulation) even though local extinctions may occur (Hanski and Simberloff 1997). Although *Z. muelleri* has a high dispersal potential (Stafford-Bell et al. 2015), it remains to be determined whether propagules of the species are dispersing within and between populations. Such events would be driving the genetic diversity and connectivity of these populations at a local scale in south eastern Australia.

Microsatellites are one of the most commonly used DNA marker in population genetics and their highly polymorphic nature can provide insights into the extent of contemporary gene flow and the resulting connectivity between far removed seagrasses populations (Kendrick et al. 2012). We obtained multi-locus microsatellite DNA genotypes for 25 populations of *Z. muelleri* to initially determine the genetic diversity of populations. Following this we aimed to determine the extent of connectivity between the populations to identify whether the present management of these sites is appropriate when viewed in light of metapopulation ecology. Gaining a greater understanding of the genetic

diversity and connectivity within populations of *Z. muelleri* in south-eastern Australia will allow for more targeted rehabilitation programs that use genetically appropriate individuals.

## Materials and Methods

### *Study sites and sampling protocols*

Samples were collected along 686 km of the Victorian coastline (22 sites in eight locations) and roughly 40 km of the east Tasmanian coast (three sites in three locations) (Fig. 1). Sampling of *Z. muelleri* occurred at low tide with collection of nine samples across a 10 m x 10 m grid from three sites within each estuary where possible (Table 1) (Arnaud-Haond et al. 2007; Inglis and Waycott 2001; supplementary material). Samples were collected at fixed points in the grid and were separated by a distance of 5 m. Volunteers collected Tasmanian samples opportunistically and due to a small seagrass population occurring within Wingan Inlet (VIC) only nine samples in total were collected in that estuary. Meristematic material containing an upright shoot with attached rhizome was removed from the sediment by hand, flushed with fresh water, pat dried with paper towel and placed in 50 ml centrifuge containers with silica crystals for later analysis. Genomic DNA was extracted from each sample using DNeasy Plant Kits (QIAGEN) following the manufacturer's instructions.

### *Genetic analyses*

We characterised the polymorphism of eleven microsatellite DNA loci using primers previously developed for *Z. muelleri* (ZosNSW02, ZosNSW18, ZosNSW19, ZosNSW20, ZosNSW23, ZosNSW28, ZosNSW34, ZosNSW38,

ZosNSW43, ZosNSW45 and ZosNSW46) (Sherman et al. 2012). The forward primer of each pair was labelled with an M13 tag (5' CACGACGTTGTAAAACGAC) on the 5' end for later use in the universal dye labelling process (Boutin-Ganache et al. 2001). Polymerase chain reactions (PCR) (20 µL) were undertaken using HotStarTaq Plus PCR Master Mix (10 µL) (QIAGEN) following manufacturer's instructions. Final concentrations of 2.4 µM of the M13 tag 5' labelled with an Applied Biosystems (ABI) dye (NED, FAM, VIC or PET), the locus-specific tailed (0.6 µM) and untailed (2.4 µM) primers, approximately 10 ng of genomic DNA were used in each PCR. PCR products were amplified in a Biorad MyCycler thermocycler using the following conditions: an initial denaturation step of 95°C for 60s followed by 35 cycles of 94°C for 45s, 53°C for 60s, 72°C for 60s with final elongation at 72°C for 5 min.

PCR product sizes were scored commercially (Australian Genome Research Facility AGRF) on the GeneMapper software (Applied Biosystems) using the GeneScan 500 Liz size standard. Samples that produced poor results or failed to amplify were re-run following the process described previously. To identify shared multilocus genotypes (MLG) within the populations, we used 'Find Clones' within GENALEX 6.5 (Peakall and Smouse 2006; Peakall and Smouse 2012; Sinclair et al. 2014)). The statistical power for properly identifying those shared MLGs was determined through calculation of the probability of identity ($P_{ID}$). Doing so allowed us to determine whether the shared MLGs were from the same vegetative clone or resulted from seed recruitment. Clonal Richness [R = (G-1)/(N-1)] was estimated for each meadow where G was the number of unique MLGs and N was the total number of plant samples. An R value of zero would indicate a single clone and a clonal richness score of 1 would indicate a

different genet for every sample (Dorken & Eckert 2001). Following identification and removal of clones from the dataset, population genetic differentiation was initially determined through estimation of variation among sampled sites with the calculation of pairwise $F_{ST}$ (Wright 1943), G'ST (Hedrick 2005) and D (Jost 2008) in GENALEX 6.5 (Peakall and Smouse 2006; Peakall and Smouse 2012; Sinclair et al. 2014). Where non-significance of $F_{ST}$ among sites within locations was identified, those sites were deemed to be part of the same gene pool and were pooled for further analysis. The following analyses were then undertaken in GENALEX 6.5 (Peakall and Smouse 2006; Peakall and Smouse 2012; Sinclair et al. 2014): the total number of alleles (Na), observed heterozygosity ($H_o$), expected heterozygosity ($H_e$), the fixation index (F) and deviation of loci from Hardy-Weinberg equilibrium (HWE). To determine the proportion of variation within the total genetic variation that could be attributed to within and among sampled populations and regions, analysis of molecular variance (AMOVA) was also performed.

Analysis of isolation by distance (IBD) was undertaken through a Mantel test to identify correlations between genetic distance ($F_{ST}$ /1- $F_{ST}$) and the oceanographic distance (km) between the populations for all sample sites, in western and central Victoria and eastern Victoria and Tasmania using GENALEX 6.5 (Peakall & Smouse 2006, 2012). While the use of particle transport models can provide a more accurate determination of oceanographic distance, the development of such a model was outside the scope of this study. Oceanographic distance was therefore calculated in QGIS 2.8.1 as the shortest distance between sampled sites (QGIS 2015).

To identify the presence of distinct genetic clusters, assign individuals to populations and identify sites of admixture, the Bayesian modelling incorporated in the program STRUCTURE 2.3.4 (Pritchard et al. 2000) was used. We performed a 100,000 burnin length and 500,000 Markov Chain Monte Carlo (MCMC) simulations for $K$ = 1-10 with 10 iterations for each $K$ to ensure consistency across all runs. This program assumes a fixed number of populations ($K$) using the Dirichlet distribution to model allele frequencies for each population and provides an estimation of the probability that an allele belongs to a particular population (Pr(X\$K$) (Frankham et al. 2002; Hartl and Clark 2007; Pritchard et al. 2000). To determine the appropriate value for $K$, we used the methods described by Evanno et al. (2005). A Principal Coordinate Analysis (PCoA) was then performed to provide further insight into the geographic relationships between each MLG and the population means.

## Results

### *Amplification of PCR products and microsatellite loci*

The number of alleles at a locus ranged from two to 14 (mean = 8, SD = 4) with a total of 76 alleles detected across all loci. Observed and expected heterozygosity ranged from 0.27–0.70 and 0.33–0.49. Significant departures from HWE were observed for two loci (ZosNSW23 and ZosNSW43) due to heterozygote deficiency and, as a result of the small number of alleles scored in ZosNSW02 and ZosNSW38, failed to complete HWE tests for these loci. All further analyses were tested with and without inclusion of ZosNSW02 and ZosNSW38. Inclusion of the loci did not significantly influence the results of the

196 analyses and so the results presented here include analyses with the inclusion
197 of ZosNSW02 and ZosNSW38.

198 The combined probability of identity for this dataset was low ($P_{ID}$ = 1.7 x $10^{-8}$)
199 indicating a high likelihood that unique MLGs were identified. As a result, clones
200 were considered to come from the same vegetative source and were removed
201 from the dataset for further population genetic analyses. Clonal diversity (R) for
202 all genotypes was high across all studied sites with 64% of the populations
203 having unique MLGs (clonal richness = 1) (Table 2). Where clones were
204 identified, they did not occur within neighbouring sample points on 40% of
205 samples, rather they occurred in a mosaic of entwined individuals. One site
206 within Port Phillip Bay (PPB2) had a high degree of clonality with 44% of
207 samples coming from the same clone. The remaining sites had on average two
208 samples coming from the same clone and there was no pattern of clonality
209 between sampled sites. Shallow Inlet was the only estuary that had a shared
210 MLG between sites (SH1 and SH2; ≈ 600 m apart) across the sampled
211 populations indicating that connectivity through vegetative recruitment is
212 occurring within these sites.

213 Three sites showed non-significant differentiation between all sites sampled
214 within the same estuaries in the initial analysis (CUI - $F_{ST}$ = 0.043, $P$ = 0.017;
215 $G'_{ST}$ = 0.034, $P$ = 0.002; $D$ = 0.029, $P$ = 0.020; SI - $F_{ST}$ = 0.015, $P$ = 0.434; $G'_{ST}$
216 = 0.025, $P$ = 0.002; $D$ = 0.016, $P$ = 0.657; CI - $F_{ST}$ = 0.025, $P$ = 0.198; $G'_{ST}$ =
217 0.018, $P$ = 0.002; $D$ = 0.012, $P$ = 0.198) (Table 2). Following pooling of the sites
218 in CUI, SI and CI, genetic differentiation among most sample sites was
219 generally high ($F_{ST}$ = 0.245, $P$ = 0.001; $G'_{ST}$ = 0.398, $P$ = 0.001; $D$ = 0.238, $P$ =

220 0.001). Non-significant differentiation was also observed between some sites
221 within Lake Tyers (LT1 and LT2 - $F_{ST}$ = 0.022, $P$ = 0.119; $G'_{ST}$ = 0.024, $P$ =
222 0.002; $D$ =0.016, $P$ = 0.163; LT2 and LT3 - $F_{ST}$ = 0.014, $P$ = 0.447; $G'_{ST}$ = 0.001,
223 $P$ = 0.002; $D$ = 0.001, $P$ = 0.469). However, as there was significant
224 differentiation between LT1 and LT3, the sites within Lake Tyers were not
225 pooled for further analysis. One site sampled within Lake Tyers (LT2) showed
226 non-significant differentiation from a site located within the Gippsland Lakes
227 (GL2) indicating that there is a degree of connectivity between the two sites ($F_{ST}$
228 = 0.032, $P$ = 0.057; $G'_{ST}$ = 0.079, $P$ = 0.062; $D$ = 0.047, $P$ = 0.062). The
229 AMOVA indicated that variation among individuals within sample sites
230 accounted for 77% of the total variation, 19% occurred among sample sites and
231 4% occurred among sample regions ($p < 0.001$).

232 Results of the Mantel test ($r^2$ = 0.137, p = 0.003) for all sites indicated a weak
233 positive relationship existed between standardized genetic distance (FST /1-
234 FST) and the oceanographic distance between sample sites (km). Separate
235 Mantel tests across the western and central Victorian showed a weak positive
236 relationship ($r^2$ = 0.093, p = 0.016) while there was a stronger relationship
237 between sites located in eastern Victoria and Tasmania ($r^2$ = 0.608, p = 0.001).

238 Assignment of individuals using STRUCTURE 2.3.4 (Pritchard et al. 2000)
239 clearly identified two distinct population clusters ($K$=2) across all sampled
240 populations (Fig. 2). When individual populations were taken into account there
241 was somewhat of a clear distinction between the central Victorian populations
242 (PPB, WP, SI, CI) and those of eastern Victoria and Tasmania (GL, LT, LSP,
243 MFMC, MCB, OR, WI). There were, however, a number of individuals placed

within the eastern Victorian genetic cluster (green lines Fig. 2) that showed similarities with those of western Victorian (red lines Fig. 2) indicating gene flow has occurred between the two clusters. This is also apparent when taking into account the placement of Curdies Inlet (far west Victoria) within the eastern cluster and Western Port and Wingan Inlet were sites of admixture between the two clusters.

Differentiation of sample sites via PCoA showed structured grouping of sample sites based on location. Similarities were observed among meadows located on the eastern and western coasts of Victoria with Tasmanian sites being closely grouped with those of eastern Victoria. Corner Inlet (CI) and Curdies Inlet (CuI) situated on the eastern and western sides of Wilsons Promontory respectively, were the only sites with a larger number of MLGs less similar to other sites based on the spread of clustering in the PCoA (Fig. 3).

## Discussion

This study aimed to determine the connectivity of populations of *Z. muelleri* in south-eastern Australia. This was achieved through analysis of genotypic diversity and connectivity of 25 populations of the species across Victoria and eastern Tasmania. We hypothesized that gene flow between regional populations would be limited, while local populations would display an important degree of connectivity. Although significant differentiation between some sites led to reduced sample sizes for some estuaries and further analysis may be warranted, our results still provide an important indicative understanding of gene flow in the region. We found a high degree of genetic diversity within the sampled populations with 64% of populations having unique MLGs (clonal

richness = 1) (Table 2) with only two sites sharing MLGs (SH1 and SH2). Genotypic diversity across all sampled sites was more variable with two sites (PPB 2 and MI) having lower levels of diversity when compared to the remaining sites (Table 2). The high degree of clonal diversity identified in the present study may be attributed to the sampling procedure used whereby each plant sample collected was separated by a distance of at least 5 m. At the scale used, meadows that contained clones were found to be a mosaic of entwined individuals rather than single genets occurring in geographical isolation from one another. Research by Jones et al. (2008), that utilised finer scale (1 m) and regional sampling of populations of *Z. muelleri* within New Zealand waters, found a similar mosaic at the fine scale used. Sites with a high degree of connectivity without impedance to gene flow were also genotypically admixtured, while far removed sites were considered to be genetically isolated from one another.

Previous studies on the relative importance of sexual versus asexual reproduction and the influence of genotypic diversity on maintaining populations of *Z. muelleri* are varied. For instance, in their study of populations of *Z. muelleri* in Lake Macquarie, New South Wales, Australia, Macreadie et al. (2014) was unable to identify a relationship between the level of genotypic diversity and the importance of sexual versus asexual reproduction. Conversely (Sherman et al. 2016) suggests that high levels of genotypic diversity was an indicator sexual reproduction in the same study location. Based on the high number of unique MLGs found within the present study, it may therefore be possible that sexual reproduction and recruitment is occurring within the study sites however, identification of seedling recruitment is required to confirm this. Regardless, it is

293 clear that high genetic diversity within seagrass populations can provide a
294 number of benefits that are relatively immediate or may occur over ecological
295 timeframes. Over the short term, seagrass populations with high genetic
296 diversity have been found to have greater growth and greater resistance and
297 resilience to disturbance (Hughes and Stachowicz 2004; Procaccini et al. 2007).
298 Furthermore, high genetic diversity within seagrass populations may also have
299 a flow-on effect to other trophic levels. For instance, increasing genetic diverisity
300 within popualtions of *Z. marina* increases both plant biomass and faunal
301 diversities (Reusch et al. 2005). Similarly, genotypically diverse meadows of *Z.*
302 *muelleli* have been found to have higher faunal abundance than genotypically
303 depauperate meadows {Macreadie, 2014 #984}. Over the longer term,
304 understanding the diversity of seagrass populations provides important
305 information for translocation experiments. The production of clones by
306 seagrasses results in the replication of positive (and potentially negative) traits
307 that may be helpful in controlling environmental influences (Procaccini et al.
308 2007). A plant that has adapted to an environmental extreme will have a greater
309 likelihood of survival when transplanted within a similar environment and may
310 allow for rapid adaptation of a population to future stressors (Bradshaw and
311 Holzapfel 2006).

312 We found shared MLGs occurred between two sites within Shallow Inlet that
313 were separated by a distance of roughly 600m. As studies of this nature are
314 only able to sample a small fraction of the genotypes in any given population,
315 the low combined probability of identity for this dataset ($P_{ID}$ = 1.7 x $10^{-8}$) was
316 expected. However, while it is also likely that this study has underestimated the
317 degree of clonal dispersal between populations, based on the shared MLGs

between the two sites within Shallow Inlet, dispersal and eventual recruitment of vegetative propagules may be occurring. Vegetative propagules have comparatively greater dispersal potential than seeds due to long term viability (>5w) and large lacunal spaces within rhizomatous tissues, which account for 45% of the internal volume (Stafford-Bell et al. 2015). The dispersal of such propagules in the order of hundreds to thousands of kilometres has previously been suggested for some *Zostera* species (Berković et al. 2014; Thomson et al. 2014). Similar trends have been observed in other marine flora, including the invasive marine alga, *Caulerpa taxifolia* (Smith and Walters 1999), and the giant kelp, *Macrocystis pyrifera* (Hernández‐Carmona et al. 2006). While we have identified only one instance of possible vegetative recruitment, our findings of non-significant differentiation within some estuaries indicate that dispersal of vegetative propagules via localised currents may play an important role in maintaining connectivity within these sites. Identifying the occurrence of further supplementation from surrounding sites could be achieved through greater in-depth phylogenetic studies, which incorporate next generation sequencing. Determining potential source and sink populations would also facilitate more targeted genetic analysis of populations.

Although genetic diversity within the studied meadows was high, we found varying degrees of differentiation between the examined *Z. muelleri* populations, which may be explained by the hydrological processes in the region. The marine waters of southern Australia, particularly within Bass Strait, are subject to a range of tidal, wind-driven and oceanic currents. Tidal currents occur simultaneously from both the west and east creating a region of reduced tidal current within central Bass Strait at the confluence of the westerly and easterly

343 tides (Keough and Black 1996). Similar to the tidal currents, there is a reduction
344 in wind-driven circulation around Port Phillip Bay and Western Port (Harrison et
345 al. 2008). Oceanic swells within the west of the region occur from the south-
346 west leading to long-shore drift in an easterly direction for up to nine months of
347 the year (Bird 2010).

348 Of particular interest in the present study was the finding that contemporary
349 oceanic and in-shore conditions may be strongly influencing the significant
350 differentiation of Corner Inlet and, to a lesser degree, Curdies Inlet from all other
351 populations. Modelling by Coller (2007, http://sahultime.monash.edu.au/)
352 indicates Corner Inlet became inundated roughly 9000 years ago following the
353 breaking of the LGM. The site is now characterised by large, shallow mudflats
354 and sandbanks with more than 40% of the tidal flats being exposed during low
355 tide (WGCMA 2013). As a result, exchange of waters within Corner Inlet takes a
356 number of tidal cycles to occur (Molloy et al. 2005). The hydrological influence
357 in the region and the slow flushing of the inlet would reduce the movement of
358 propagules both into and out of Corner Inlet. High connectivity of populations
359 within Corner Inlet, however, is likely based on our results of non-significant
360 differentiation between meadows within the inlet (Table 3) and previous
361 numerical modelling that investigated the potential dispersal of *P. australis* in
362 Corner Inlet (Sinclair et al. 2016). The role of contemporary oceanic barriers in
363 isolating populations has previously been investigated by Cowen et al. (2006)
364 who found self-recruitment of reef fishes accounted for roughly 57% of all
365 recruitment events for populations in close proximity to the semi-permanent
366 Panama-Columbia Gyre. Furthermore, hydrology within the Gulf of Maine has

resulted in restricted population connectivity of the benthic amphipod *Corophium volutator* (Einfeldt and Addison 2013).

Understanding how gene flow can influence *Z. muelleri* populations within Corner Inlet is an important step in conserving them. The differentiation observed in the present study indicates the exchange of genetic material between this and surrounding sites has historically been low. Genetic clustering in the region however has identified that two meadows within Corner Inlet were closely correlated with Tasmanian coastal meadows (Sinclair et al. 2016). Greater in-depth genetic analysis at the site may further elucidate the connectivity within between Corner Inlet and surrounding populations, such as those within Tasmania.

The significant differentiation of Curdies Inlet from the remaining sites in the current study may also be the result of contemporary currents in the region. Reverse hydrodynamic modelling undertaken to determine potential spawning grounds of King George whiting (*Sillaginodes punctata*) has shown the influence of these currents with spawning locations occurring some 400km to the west of the eventual recruitment site of Port Phillip Bay (Jenkins et al. 2000). It is therefore likely that the populations within Curdies Inlet may, in fact, be more closely related to populations located to the west of the site. Identification and genetic analysis of such populations would elucidate this question.

When considered in the light of metapopulation ecology, with the exception of SH1 and SH2, all of the populations within the present study may be deemed to be fragmented with little to no exchange of propagules with surrounding sites. Fragmentation, and therefore isolation of these population may negatively

391 impact the species and its associated biota that may include habitat loss,
392 reduced population sizes and increased genetic isolation (Aguilar et al. 2008).
393 Given the complexity of habitat fragmentation processes, it is often difficult to
394 identify clear species response patterns. However, the majority of studies have
395 identified habitat fragmentation as a major cause of reduced genetic diversity
396 (Aguilar et al. 2008). Should the lack of immigration from surrounding
397 populations identified in the present study continue, resilience of those
398 populations to disturbance may be greatly reduced (Aguilar et al. 2008;
399 Procaccini et al. 2007).

400 The studied populations of *Z. muelleri* within south-eastern Australia exist in an
401 environment influenced by both historical and contemporary processes, factors
402 that must be taken into account when considering their appropriate
403 management. They may be characterised as having high clonal diversity, are
404 strongly differentiated and generally exist in isolation from one another at the
405 regional scale. At the local level, however, non-significant, within-estuary
406 differentiation indicates that contemporary conditions are allowing the dispersal
407 and recruitment of propagules from surrounding sites.

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Fig. 1. Location of *Zostera muelleri* populations sampled within south-eastern Australia. Sites were located within a) Victoria (Curdies Inlet (CuI), Port Phillip Bay (PPB), Western Port (WP), Shallow Inlet (SI), Corner Inlet (CI), Gippsland Lakes (GL), Lake Tyers (LT), Wingan Inlet (WI) and b) Tasmania (Orford (OR), Maria Island Four Mile Creek (MFMC), Maria Island Chainman's Bay (MCB), Little Swanport Estuary (LSP). Note: following identification of clones, sites within Maria Island were pooled.

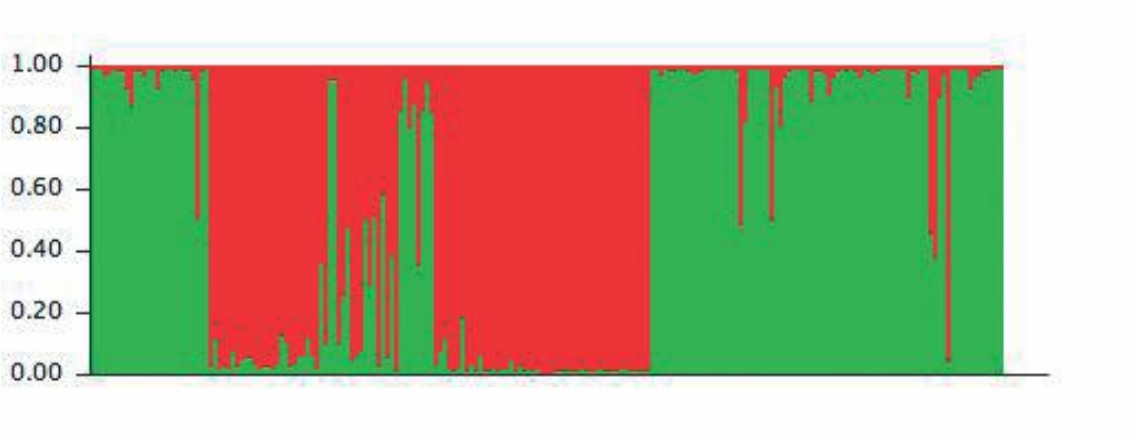


Fig. 2. Population clusters within the twelve *Zostera muelleri* populations as defined by STRUCTURE 2.3.4. Individual samples are represented by a single vertical line, broken into coloured segments for each *K*. Lengths of each colour are proportional to each of the *K* inferred clusters.

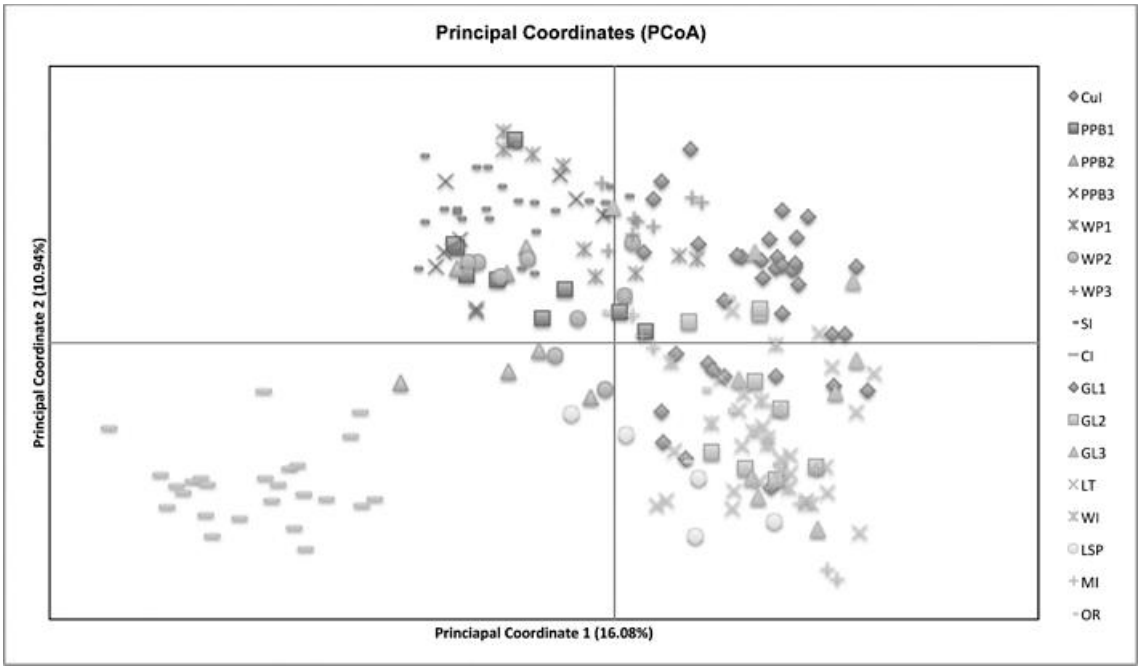


Fig. 3. Principal coordinates analysis (PCoA) indicating the spatial separation of MLGs of the *Zostera muelleri* sample sites. Refer to Table 1 for population name abbreviations.

Table 1: Sampled populations of Z. muelleri for microsatellite analysis. Sites are located within Victoria (CuI, PPB, WP, SI, CI, GL, LT, WI) and Tasmania (OR, MFMC, MCB, LSP). Victorian sites are ordered from west coast to east coast populations.

| Site | Abb. | Form | Classification | Entrance orientation | Intertidal area ($km^2$) | Water area ($km^2$) | Entrance form | Entrance width (km) | Mean wave height (m) | Mean wave period (s) | Tidal range (m) | Tide type |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Curdies Inlet | CuI | Estuary | Wave dominated | S | 0.24 | 2.94 | Single | 0.13 | 2.3 | 8.9 | 0.9 | Diurnal |
| Port Phillip Bay | PPB | Estuary | Tide dominated | SW | 14.1 | 1897 | Single | 3.46 | 0.61 | 6.7 | 1.2 | Semi Diurnal |
| Western Port | WP | Estuary | Tide dominated | SW/SE | 90.6 | 469 | Double | 4.87 | 1.4 | 8.3 | 2.3 | Semi Diurnal |
| Shallow Inlet | SI | Estuary | Wave dominated | SW | 7.05 | 5.03 | Single | 0.29 | 1.6 | 8.4 | 2.1 | Semi Diurnal |
| Corner Inlet | CI | Estuary | Tide dominated | SE | 387 | 378 | Single | 1.89 | 0.34 | 4.8 | 2.3 | Semi Diurnal |
| Gippsland Lakes | GL | Estuary | Wave dominated | SE | 0 | 486 | Single | 0.36 | 0.52 | 5.8 | 0.9 | Semi Diurnal |
| Lake Tyers | LT | Estuary | Wave dominated | S | 1.29 | 13.1 | Single | 0.14 | 0.91 | 5.8 | 0.9 | Diurnal |
| Wingan Inlet | WI | Estuary | Wave dominated | SSE | 0.38 | 1.5 | Single | 0.12 | 1.6 | 6.7 | 1.1 | Diurnal |
| Orford | OR | Estuary | Wave dominated | SE | 0.29 | 0.19 | Single | 0.06 | 0.61 | 5.3 | 1.1 | Diurnal |
| Maria Island Four Mile Creek | MFMC | Beach | Wave dominated | NW | 0 | NA | Single | 0.4 | 0.5 | 10 | 1 | Semi Diurnal |
| Maria Island Chinaman's Bay | MCB | Beach | Wave dominated | SW | 0 | NA | Single | 0.25 | 0.1 | 10 | 1 | Semi Diurnal |
| Little Swanport | LSP | Estuary | Wave dominated | E | 0.14 | 4.28 | Single | 0.39 | 0.5 | 5.4 | 1.2 | Diurnal |

Table 2. Sampled populations of *Z. muelleri* for microsatellite analysis where: N is the number of samples, MLG is the number of unique multilocus genotypes, R is the clonal diversity where R = (MLG-1)/(N-1), Na is the number of alleles, $H_o$ is the observed heterozygosity, $H_e$ is the expected heterozygosity and F is the fixation index.

| Site | State | Abbrev. | N | MLG | R | Na | Ho | He | F |
|---|---|---|---|---|---|---|---|---|---|
| Curdies Inlet | VIC | CuI | 27 | 27 | 1.00 | 4 | 0.490 | 0.409 | -0.177 |
| Port Phillip Bay | VIC | PPB1 | 9 | 9 | 1.00 | 3 | 0.494 | 0.397 | -0.249 |
| Port Phillip Bay | VIC | PPB2 | 9 | 7 | 0.75 | 3 | 0.587 | 0.417 | -0.412 |
| Port Phillip Bay | VIC | PPB3 | 9 | 9 | 1.00 | 3 | 0.519 | 0.447 | -0.161 |
| Western Port | VIC | WP1 | 9 | 9 | 1.00 | 3 | 0.469 | 0.442 | -0.050 |
| Western Port | VIC | WP2 | 9 | 9 | 1.00 | 3 | 0.580 | 0.473 | -0.208 |
| Western Port | VIC | WP3 | 8 | 8 | 1.00 | 2 | 0.528 | 0.336 | -0.529 |
| Shallow Inlet | VIC | SI | 27 | 25 | 0.92 | 3 | 0.702 | 0.474 | -0.495 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Corner Inlet | VIC | CI | 27 | 25 | 0.92 | 3 | 0.609 | 0.489 | -0.252 |
| Gippsland Lakes | VIC | GL1 | 9 | 9 | 1.00 | 4 | 0.531 | 0.479 | -0.118 |
| Gippsland Lakes | VIC | GL2 | 9 | 9 | 1.00 | 4 | 0.543 | 0.486 | -0.112 |
| Gippsland Lakes | VIC | GL3 | 9 | 9 | 1.00 | 4 | 0.531 | 0.486 | -0.080 |
| Lake Tyers | VIC | LT1 | 9 | 9 | 1.00 | 4 | 0.432 | 0.451 | 0.111 |
| Lake Tyers | VIC | LT2 | 9 | 9 | 1.00 | 4 | 0.444 | 0.459 | 0.044 |
| Lake Tyers | VIC | LT3 | 9 | 9 | 1.00 | 4 | 0.506 | 0.444 | -0.142 |
| Wingan Inlet | VIC | WI | 10 | 9 | 0.89 | 3 | 0.506 | 0.422 | -0.231 |
| Little Swanport | TAS | LSP | 5 | 5 | 1.00 | 2 | 0.489 | 0.382 | -0.225 |
| Maria Island | TAS | MI | 10 | 7 | 0.67 | 2 | 0.270 | 0.334 | 0.165 |
| Orford | TAS | OR | 5 | 5 | 1.00 | 2 | 0.600 | 0.447 | -0.317 |

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Table 3: Pairwise means of genetic differentiation between the 25 sampled *Z. muelleri* sample populations (FST figures are below the diagonal; Jost's D are above the diagonal. Refer to Fig. 2 for the location of each population and Table 1 for population name abbreviations.

| | **CuI** | **PPB** | **WP** | **SI** | **CI** | **GL** | **LT** | **WI** | **LSP** | **MI** | **OR** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **CuI** | | 0.216 | 0.157 | 0.210 | 0.513 | 0.150 | 0.140 | 0.179 | 0.285 | 0.289 | 0.313 | **CuI** |
| **PPB** | 0.132 | | 0.084 | 0.128 | 0.294 | 0.192 | 0.221 | 0.205 | 0.228 | 0.362 | 0.305 | **PPB** |
| **WP** | 0.087 | 0.052 | | 0.123 | 0.377 | 0.135 | 0.180 | 0.195 | 0.239 | 0.295 | 0.200 | **WP** |
| **SI** | 0.129 | 0.071 | 0.068 | | 0.368 | 0.251 | 0.273 | 0.334 | 0.306 | 0.367 | 0.331 | **SI** |
| **CI** | 0.260 | 0.160 | 0.190 | 0.184 | | 0.471 | 0.435 | 0.467 | 0.400 | 0.519 | 0.527 | **CI** |
| **GL** | 0.076 | 0.089 | 0.065 | 0.108 | 0.198 | | 0.044 | 0.085 | 0.125 | 0.229 | 0.167 | **GL** |
| **LT** | 0.079 | 0.118 | 0.092 | 0.135 | 0.207 | 0.033 | | 0.074 | 0.137 | 0.185 | 0.206 | **LT** |
| **WI** | 0.113 | 0.127 | 0.107 | 0.176 | 0.242 | 0.055 | 0.056 | | 0.146 | 0.204 | 0.213 | **WI** |
| **LSP** | 0.192 | 0.168 | 0.157 | 0.186 | 0.236 | 0.084 | 0.104 | 0.125 | | 0.222 | 0.251 | **LSP** |
| **MI** | 0.181 | 0.207 | 0.163 | 0.205 | 0.267 | 0.114 | 0.111 | 0.145 | 0.174 | | 0.268 | **MI** |
| **OR** | 0.172 | 0.156 | 0.115 | 0.158 | 0.240 | 0.087 | 0.119 | 0.134 | 0.159 | 0.171 | | **OR** |