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

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14

15 **Abstract**

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

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

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

### 35 **Introduction**

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

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

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

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

76 recruitment (Di Carlo et al. 2005; Thomson et al. 2014). Furthermore, given  
77 flowering in seagrasses is limited to a very small proportion of the population ( $\approx$   
78 10%), the low ability of seeds to disperse and high mortality of seedlings  
79 (roughly 2% of seedlings will survive past the first year) (Hemminga and Duarte  
80 2000), diversity of populations could be expected to be low. Should recruitment  
81 occur, immigrating genetic individuals supplement the genetic diversity within  
82 populations leading to an increased resilience of those populations to  
83 disturbance (Procaccini et al. 2007; Sherman et al. 2016). Maintenance of  
84 genetic diversity and supplementation of populations from surrounding sources  
85 therefore allows the persistence of a group of populations within a given area (a  
86 metapopulation) even though local extinctions may occur (Hanski and  
87 Simberloff 1997). Although *Z. muelleri* has a high dispersal potential (Stafford-  
88 Bell et al. 2015), it remains to be determined whether propagules of the species  
89 are dispersing within and between populations. Such events would be driving  
90 the genetic diversity and connectivity of these populations at a local scale in  
91 south eastern Australia.

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

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

## 104 **Materials and Methods**

### 105 *Study sites and sampling protocols*

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

### 120 *Genetic analyses*

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

124 ZosNSW43, ZosNSW45 and ZosNSW46) (Sherman et al. 2012). The forward  
125 primer of each pair was labelled with an M13 tag (5'  
126 CACGACGTTGTAAAACGAC) on the 5' end for later use in the universal dye  
127 labelling process (Boutin-Ganache et al. 2001). Polymerase chain reactions  
128 (PCR) (20  $\mu$ L) were undertaken using HotStarTaq Plus PCR Master Mix (10  $\mu$ L)  
129 (QIAGEN) following manufacturer's instructions. Final concentrations of 2.4  $\mu$ M  
130 of the M13 tag 5' labelled with an Applied Biosystems (ABI) dye (NED, FAM,  
131 VIC or PET), the locus-specific tailed (0.6  $\mu$ M) and untailed (2.4  $\mu$ M) primers,  
132 approximately 10 ng of genomic DNA were used in each PCR. PCR products  
133 were amplified in a Biorad MyCycler thermocycler using the following  
134 conditions: an initial denaturation step of 95°C for 60s followed by 35 cycles of  
135 94°C for 45s, 53°C for 60s, 72°C for 60s with final elongation at 72°C for 5 min.

136 PCR product sizes were scored commercially (Australian Genome Research  
137 Facility AGRF) on the GeneMapper software (Applied Biosystems) using the  
138 GeneScan 500 Liz size standard. Samples that produced poor results or failed  
139 to amplify were re-run following the process described previously. To identify  
140 shared multilocus genotypes (MLG) within the populations, we used 'Find  
141 Clones' within GENALEX 6.5 (Peakall and Smouse 2006; Peakall and Smouse  
142 2012; Sinclair et al. 2014)). The statistical power for properly identifying those  
143 shared MLGs was determined through calculation of the probability of identity  
144 ( $P_{ID}$ ). Doing so allowed us to determine whether the shared MLGs were from  
145 the same vegetative clone or resulted from seed recruitment. Clonal Richness  
146 [ $R = (G-1)/(N-1)$ ] was estimated for each meadow where G was the number of  
147 unique MLGs and N was the total number of plant samples. An R value of zero  
148 would indicate a single clone and a clonal richness score of 1 would indicate a

149 different genet for every sample (Dorken & Eckert 2001). Following identification  
150 and removal of clones from the dataset, population genetic differentiation was  
151 initially determined through estimation of variation among sampled sites with the  
152 calculation of pairwise  $F_{ST}$  (Wright 1943),  $G'_{ST}$  (Hedrick 2005) and  $D$  (Jost  
153 2008) in GENALEX 6.5 (Peakall and Smouse 2006; Peakall and Smouse 2012;  
154 Sinclair et al. 2014). Where non-significance of  $F_{ST}$  among sites within locations  
155 was identified, those sites were deemed to be part of the same gene pool and  
156 were pooled for further analysis. The following analyses were then undertaken  
157 in GENALEX 6.5 (Peakall and Smouse 2006; Peakall and Smouse 2012;  
158 Sinclair et al. 2014): the total number of alleles ( $N_a$ ), observed heterozygosity  
159 ( $H_o$ ), expected heterozygosity ( $H_e$ ), the fixation index ( $F$ ) and deviation of loci  
160 from Hardy-Weinberg equilibrium (HWE). To determine the proportion of  
161 variation within the total genetic variation that could be attributed to within and  
162 among sampled populations and regions, analysis of molecular variance  
163 (AMOVA) was also performed.

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



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

## 186 **Results**

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

188 The number of alleles at a locus ranged from two to 14 (mean = 8, SD = 4) with  
189 a total of 76 alleles detected across all loci. Observed and expected  
190 heterozygosity ranged from 0.27–0.70 and 0.33–0.49. Significant departures  
191 from HWE were observed for two loci (ZosNSW23 and ZosNSW43) due to  
192 heterozygote deficiency and, as a result of the small number of alleles scored in  
193 ZosNSW02 and ZosNSW38, failed to complete HWE tests for these loci. All  
194 further analyses were tested with and without inclusion of ZosNSW02 and  
195 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 ( $P_{ID} = 1.7 \times 10^{-8}$ )  
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;  $\approx 600$  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 ( $F_{ST} / 1 -$   
234  $F_{ST}$ ) 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

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

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

## 257 **Discussion**

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

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

282 Previous studies on the relative importance of sexual versus asexual  
283 reproduction and the influence of genotypic diversity on maintaining populations  
284 of *Z. muelleri* are varied. For instance, in their study of populations of *Z. muelleri*  
285 in Lake Macquarie, New South Wales, Australia, Macreadie et al. (2014) was  
286 unable to identify a relationship between the level of genotypic diversity and the  
287 importance of sexual versus asexual reproduction. Conversely (Sherman et al.  
288 2016) suggests that high levels of genotypic diversity was an indicator sexual  
289 reproduction in the same study location. Based on the high number of unique  
290 MLGs found within the present study, it may therefore be possible that sexual  
291 reproduction and recruitment is occurring within the study sites however,  
292 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 diversity  
300 within populations of *Z. marina* increases both plant biomass and faunal  
301 diversities (Reusch et al. 2005). Similarly, genotypically diverse meadows of *Z.*  
302 *muelleri* 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 ( $P_{ID} = 1.7 \times 10^{-8}$ ) 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

318 between the two sites within Shallow Inlet, dispersal and eventual recruitment of  
319 vegetative propagules may be occurring. Vegetative propagules have  
320 comparatively greater dispersal potential than seeds due to long term viability  
321 (>5w) and large lacunal spaces within rhizomatous tissues, which account for  
322 45% of the internal volume (Stafford-Bell et al. 2015). The dispersal of such  
323 propagules in the order of hundreds to thousands of kilometres has previously  
324 been suggested for some *Zostera* species (Berković et al. 2014; Thomson et al.  
325 2014). Similar trends have been observed in other marine flora, including the  
326 invasive marine alga, *Caulerpa taxifolia* (Smith and Walters 1999), and the giant  
327 kelp, *Macrocystis pyrifera* (Hernández - Carmona et al. 2006). While we have  
328 identified only one instance of possible vegetative recruitment, our findings of  
329 non-significant differentiation within some estuaries indicate that dispersal of  
330 vegetative propagules via localised currents may play an important role in  
331 maintaining connectivity within these sites. Identifying the occurrence of further  
332 supplementation from surrounding sites could be achieved through greater in-  
333 depth phylogenetic studies, which incorporate next generation sequencing.  
334 Determining potential source and sink populations would also facilitate more  
335 targeted genetic analysis of populations.

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



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

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

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

387 When considered in the light of metapopulation ecology, with the exception of  
388 SH1 and SH2, all of the populations within the present study may be deemed to  
389 be fragmented with little to no exchange of propagules with surrounding sites.  
390 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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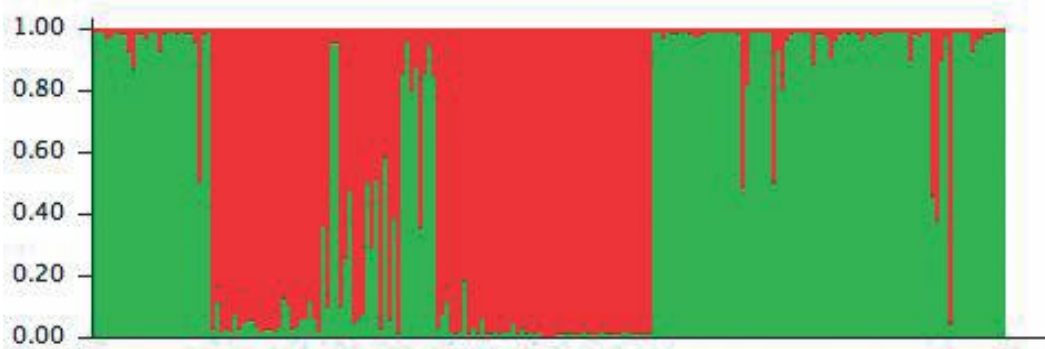
633 Fig. 1. Location of *Zostera muelleri* populations sampled within south-eastern  
634 Australia. Sites were located within a) Victoria (Curdies Inlet (CUI), Port Phillip  
635 Bay (PPB), Western Port (WP), Shallow Inlet (SI), Corner Inlet (CI), Gippsland  
636 Lakes (GL), Lake Tyers (LT), Wingan Inlet (WI) and b) Tasmania (Orford (OR),  
637 Maria Island Four Mile Creek (MFMC), Maria Island Chainman's Bay (MCB),  
638 Little Swanport Estuary (LSP). Note: following identification of clones, sites  
639 within Maria Island were pooled.

640

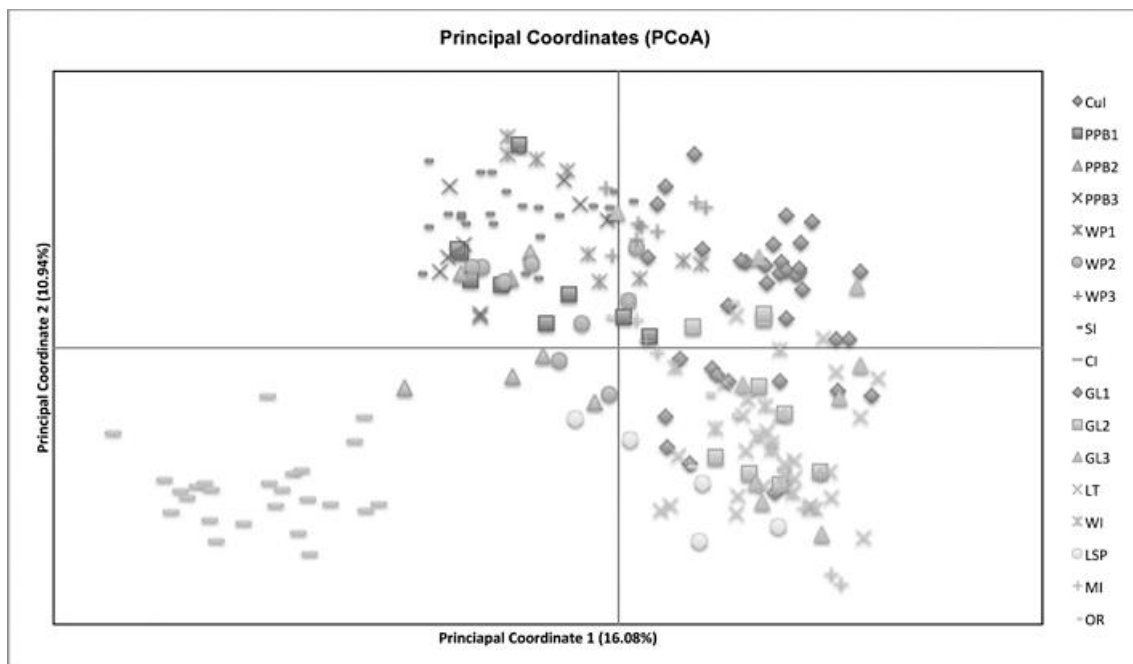
641



## Connectivity of *Zostera muelleri*



642 Fig. 2. Population clusters within the twelve *Zostera muelleri* populations as  
643 defined by STRUCTURE 2.3.4. Individual samples are represented by a single  
644 vertical line, broken into coloured segments for each *K*. Lengths of each colour  
645 are proportional to each of the *K* inferred clusters.  
646



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

653

Connectivity of *Zostera muelleri*

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

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

658

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

664

Site	State	Abbrev.	N	MLG	R	Na	Ho	He	F
Curdies Inlet	VIC	Cul	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

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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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Connectivity of *Zostera muelleri*

670 Table 3: Pairwise means of genetic differentiation between the 25 sampled *Z. muelleri* sample populations (FST figures are below  
 671 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  
 672 abbreviations.

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

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