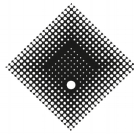

Connectivity of the seagrass Zostera muelleri within south-eastern Australia

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

3 R.E. Stafford-Bell[a*], W.F.D. van Dongen[b], R.W. Robinson[b], A.A. Chariton[c]

4 [a] Department of Jobs, Precincts and Regions, Victoria, Australia

5 [b] Institute for Sustainable Industries and Liveable Cities, Victoria University, Victoria,
6 Australia

7 [c] Department of Biological Sciences, Macquarie University, New South Wales, Australia

8
9 **Address** [a] : 475 Mickleham Rd**,** Attwood Victoria 3049, Australia
10
11 **Address** [b] : PO Box 14428, Melbourne Victoria 8001, Australia
12
13 **Address** [c] : Macquarie University, NSW 2109, Australia
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

[*]Corresponding author: richard.stafford-bell@ecodev.vic.gov.au

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 (≈2mm) 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 (≈
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 µL) were undertaken using HotStarTaq Plus PCR Master Mix (10 µL)
129 (QIAGEN) following manufacturer's instructions. Final concentrations of 2.4 µM
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 µM) and untailed (2.4 µM) 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 (Na), 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; ≈ 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

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 (CuI)
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 admixtured,
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 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 ($P_{ID} = 1.7 \times 10^{-8}$) 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 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

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.

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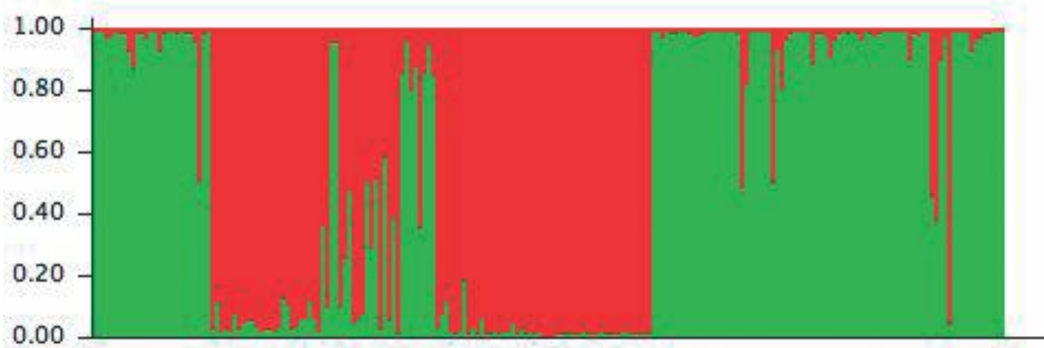

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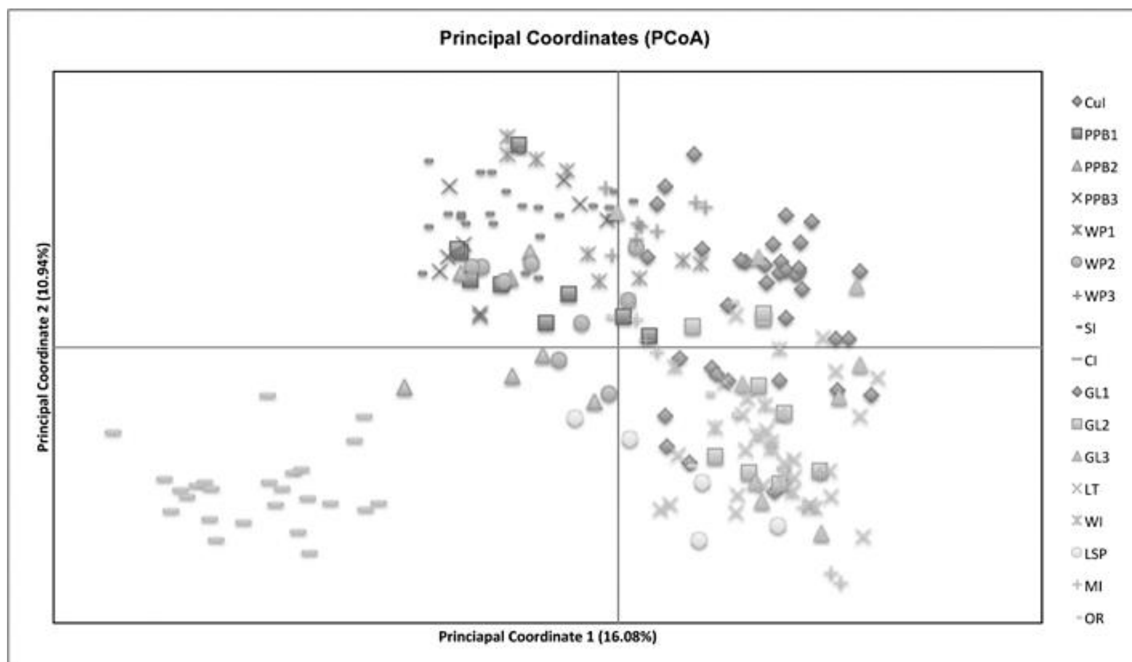
643 Fig. 2. Population clusters within the twelve *Zostera muelleri* populations as
644 defined by STRUCTURE 2.3.4. Individual samples are represented by a single
645 vertical line, broken into coloured segments for each *K.* Lengths of each colour
646 are proportional to each of the *K* inferred clusters.



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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

654 Table 1: Sampled populations of Z. muelleri for microsatellite analysis. Sites are
655 located within Victoria (CuI, 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^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	Semi 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

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	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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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.

	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**

673