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**Contrasting patterns of population connectivity between regions in a commercially important mollusc *Haliotis rubra*: integrating population genetics, genomics and marine LIDAR data.**

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32

33 **Abstract**

34 Estimating contemporary genetic structure and population connectivity in marine  
35 species is challenging, often compromised by genetic markers that lack adequate  
36 sensitivity, and unstructured sampling regimes. We show how these limitations can be  
37 overcome via the integration of modern genotyping methods and sampling designs  
38 guided by LIDAR and SONAR datasets. Here we explore patterns of gene flow and  
39 local genetic structure in a commercially harvested abalone species (*Haliotis rubra*)  
40 from South Eastern Australia, where the viability of fishing stocks is believed to be  
41 dictated by recruitment from local sources. Using a panel of microsatellite and  
42 genome-wide SNP markers we compare allele frequencies across a replicated  
43 hierarchical sampling area guided by bathymetric LIDAR imagery. Results indicate  
44 high levels of gene flow and no significant genetic structure within or between  
45 benthic reef habitats across 1400 km of coastline. These findings differ to those  
46 reported for other regions of the fishery indicating that larval supply is likely to be  
47 spatially variable, with implications for management and long-term recovery from  
48 stock depletion. The study highlights the utility of suitably designed genetic markers  
49 and spatially informed sampling strategies for gaining insights into recruitment  
50 patterns in benthic marine species, assisting in conservation planning and sustainable  
51 management of fisheries.

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58 **Introduction**

59 Estimating contemporary genetic structure and population connectivity in marine  
60 species can be challenging. Population sizes are often large and the degree of  
61 connectivity between populations is often high compared with species from  
62 freshwater and terrestrial systems ([Allendorf \*et al.\* 2010](#); [Waples 1998](#)). These factors  
63 tend to drive high levels of genetic variation and suppress drift processes and rates of  
64 genetic differentiation ([Hauser & Carvalho 2008](#); [Whitlock & McCauley 1999](#)).  
65 Because of these demographic characteristics, it is believed that insufficient time has

66 passed for many temperate marine species to reach migration-drift equilibrium  
67 following the colonization of post-glacial habitats ([Hauser & Carvalho 2008](#); [Hewitt](#)  
68 [2004](#)). Estimates of contemporary population connectivity based on genetic markers  
69 might then be inaccurate with the potential to mislead management. Despite these  
70 perceived limitations, modern genotyping methods (e.g. large panels of microsatellite  
71 and Single Nucleotide Polymorphism, SNP, markers) can provide adequate power for  
72 detecting patterns of genetic differentiation on small spatial scales in marine systems  
73 ([Benestan et al. 2015](#); [Teske et al. 2015b](#)), as long as adequately replicated sampling  
74 designs and appropriate analytical approaches are used ([Hauser & Carvalho 2008](#)).

75 Sampling in the marine environment can be logistically difficult, and is often  
76 opportunistic and at scales that are sub-optimal for detecting local genetic structure.  
77 Several studies have found evidence of local genetic structuring when using replicated  
78 hierarchical sampling designs at local spatial scales, overruling findings of panmixia  
79 based on broad geographic sampling ([Hoffmann et al. 2012](#); [Miller et al. 2009](#);  
80 [Viricel & Rosel 2014](#)). Hierarchical designs provide an opportunity to target small  
81 local populations that are more likely to be at migration-drift equilibrium and  
82 susceptible to drift processes ([Allendorf et al. 2010](#); [Whitlock & McCauley 1999](#)),  
83 and the ability to directly test dispersal limitations through relatedness based analyses  
84 (i.e. spatial autocorrelation analyses, assignment tests). Such analyses can  
85 complement and sometimes overcome the limitations of traditional measures of  
86 population differentiation such as  $F_{ST}$ , where weak estimates can be misleading and  
87 influenced by statistical artifacts ([Waples 1998](#)). However, designing suitable  
88 sampling regimes remains a challenge for many marine species, particularly benthic  
89 species whose population structure is dependent on habitat structure (i.e. complexity,  
90 extent and connectedness), which can be difficult to measure ([Ierodiaconou et al.](#)  
91 [2007](#); [Ierodiaconou et al. 2011](#)).

92 Recent advances in geospatial science now provide new opportunities for  
93 assessing benthic habitat structure on local and regional scales, such as LIDAR and  
94 SONAR technologies that are being used increasingly for imaging seabeds around the  
95 world ([Brown et al. 2011](#); [Lecours et al. 2015](#); [Young et al. 2015](#)). High-resolution  
96 geo-physical datasets (with precision of up to one metre) are being used to describe  
97 the heterogeneity of the benthic habitats (spatial arrangement, structural  
98 characteristics and connectedness) and to explore ecosystem dynamics at both fine  
99 and broad geographical scales ([Jalali et al. 2015](#)). This information should guide

100 spatial sampling efforts across habitats and environmental gradients for genetic  
101 studies, and help identify habitat features that influence patterns of genetic structure.  
102 Marine molluscs of the family Haliotidae, commonly referred to as abalone, are a  
103 group of benthic reef species that have been targeted by commercial fisheries in  
104 eleven countries, forming an important global industry worth approximately \$US180  
105 million ([Gordon & Cook 2013](#)). Many abalone fisheries have collapsed in recent  
106 decades due to over exploitation, environmental change and disease, with a number of  
107 target species now listed as endangered or considered 'species of concern' ([Gruenthal](#)  
108 [& Burton 2005](#); [Hauck & Sweijd 1999](#); [Kashiwada & Taniguchi 2007](#); [Leiva &](#)  
109 [Castilla 2001](#)). Despite reduced fishing pressure and the closure of many abalone  
110 fisheries, stock recovery appears to be slow and absent in many cases ([Karpov et al.](#)  
111 [2000](#)). Ecological studies indicated that larval dispersal is likely to be limited for  
112 many abalone species, contributing to slow recovery ([McShane et al. 1988](#); [Prince et](#)  
113 [al. 1987, 1988](#)). However these studies were limited to a single species and only two  
114 geographic regions in southern Australia. Furthermore the studies of Prince *et al.*  
115 (1987, 1988) were limited to low relief boulder habitats in sheltered bays.  
116 Consequently there is a lack of evidence that spans different species, habitat types and  
117 fishing jurisdictions, and from high-energy sea conditions that typify productive  
118 abalone habitat.

119 Genetic studies of various *Haliotis* species have provided evidence of  
120 population subdivision along various coastlines of the world ([Chambers et al.](#)  
121 [2006](#); [Gruenthal et al. 2007](#); [Gruenthal & Burton 2008](#); [Miller et al. 2014](#); [Piggott et](#)  
122 [al. 2008](#)). In contrast, low levels of genetic structuring and even cases of panmixia  
123 have been reported for some abalone species, suggesting larval movement may  
124 depend on species and/or geography ([Bester-van der Merwe et al. 2011](#); [Brown](#)  
125 [1991](#); [Coates et al. 2014](#); [Gutierrez-Gonzalez et al. 2007](#); [Li et al. 2006](#)). However,  
126 the number of genetic loci in previous studies have often been relatively low, and  
127 interpretations about population structure based on some of the loci may have been  
128 affected by selection and the presence of null alleles. In addition, sampling of abalone  
129 has often been at scales that are sub-optimal for detecting local genetic structure.  
130 Nevertheless, the most comprehensive studies that have included replicated spatial  
131 sampling efforts at local spatial scales provide evidence in support of metapopulation  
132 structure and a dependency of self-recruitment at the reef level in some *Haliotis*  
133 species ([Miller et al. 2009](#); [Miller et al. 2014](#); [Temby et al. 2007](#)).

134 In the Southern Ocean of Australia, the world's largest wild abalone fisheries  
135 target *H. rubra*, in five states extending from Western Australia to southern New  
136 South Wales and Tasmania, with a net value of US\$79 million ([Mundy et al. 2014](#)).  
137 Here the marine physical environment is highly variable, driven by converging ocean  
138 currents, strong environmental gradients, habitat discontinuities, and varying degrees  
139 of exposure to wave energy (Miller et al. 2013; Rattray et al. 2015; Ridgway and  
140 Godfrey 1997; Sandery and Kämpf 2007). Heterogeneity of these physical factors is  
141 particularly pronounced in Victorian coastal waters in South Eastern Australia, an  
142 area also influenced by dramatic sea-level changes driven by historical glacial cycling  
143 (Lambeck & Chappell 2001). The Victorian *H. rubra* fishery is divided into three  
144 commercial fishing Zones (Western, Central and Eastern), two of which have suffered  
145 major declines in recent years due to disease (Abalone Viral Ganglioneuritis;  
146 [Mayfield et al. 2011](#)), and invasive urchin species (*Centrostephanus rodgersii*; [Ling](#)  
147 [2008](#)). Genetic information can assist the management and recovery of Victorian  
148 fisheries, as current patterns of stock connectivity are uncertain. However, current  
149 management of these fisheries assumes that stock viability is dictated by recruitment  
150 from local sources. Miller et al. ([2009](#)) found low genetic subdivision across reefs  
151 ( $F_{ST} = 0.009$ ,  $F'_{ST} = 0.027$ ) and little structure at higher levels across regions in the  
152 Tasmanian *H. rubra* fishery, suggesting that abalone populations are largely self-  
153 recruiting. However, these patterns may not extend to other areas given the variable  
154 hydrodynamics (including current intensity and direction, wave exposure, etc.) and  
155 physical and ecological benthic structure of the Southern Australian coastline  
156 ([Ierodiaconou et al. 2007](#); [Ierodiaconou et al. 2011](#); [Sandery & Kaempf 2007](#)). It is  
157 possible that planktonic larval movement (the primary dispersal phase lasting up to 10  
158 days; [Hahn 1988](#)) and recruitment success will be spatially variable across *H. rubra*  
159 fisheries, as has been observed for other marine species, including molluscs ([Cowen](#)  
160 [et al. 2006](#); [Gilg & Hilbish 2003](#); [Morgan et al. 2000](#)).

161 In this paper we integrate modern genotyping methods with high-resolution  
162 bathymetric data to explore patterns of genetic structure in Victorian *H. rubra*  
163 fisheries; and to determine if patterns differ across fisheries due to geographic effects.  
164 Due to issues associated with genetic markers in previous *Haliotis* genetic research,  
165 we use a selected panel of microsatellite loci and high-density Single Nucleotide  
166 Polymorphism (SNP) markers from reduced representation genome sequencing. We  
167 take advantage of unique high-resolution bathymetry maps of Victorian coastal waters

168 to guide a replicated hierarchical sampling regime. This allows for sensitive analyses  
169 of localized recruitment in populations expected to have low effective population  
170 sizes and likely to be at migration-drift equilibrium, as well as genetic patterns across  
171 and within reefs in Victorian fisheries at a wide range of spatial scales. Our findings  
172 stand in sharp contrast with those from Tasmania, and we discuss the implications for  
173 fisheries management, as well as the benefits of integrating modern genotyping and  
174 geospatial technologies in marine systems.

175

176

## 177 **Methods**

### 178 *Sample collections*

#### 179 Broad geographic sampling

180 Sampling across the Victorian Western, Central and Eastern Zone fisheries was  
181 conducted to determine spatial patterns of stock connectivity and genetic diversity at a  
182 large spatial scale (Figure 1; Appendix 1, see Supporting Information). *Haliotis rubra*  
183 tissue samples from the Western Zone fishery were provided by the Department of  
184 Economic Development, Jobs, Transport, and Resources (DEDJTR), Victoria, for  
185 genetic analysis. A total of 1264 whole specimens, from eight sites representing  
186 Western Zone reef codes (The Craggs, The Water Tower, Murrells, Inside Cape  
187 Nelson, Levy Point, South Bridgewater, and Whites Beach) and 10 size classes (50  
188 mm to 149 mm), were collected in 2009 by DEDJTR. Specimens from each site were  
189 collected within a 25 m radius of an anchored vessel, and subsequently preserved at -  
190 20 ° C. From these samples, we collected 50 mg biopsies from 320 individuals (40 per  
191 site), representing each of the reef codes and size classes, for genetic analysis.

192 Dissection tools were sterilised between samples to avoid cross contamination. The  
193 biopsied material was transferred to 2 ml microcentrifuge tubes containing 100%  
194 ethanol and transported to the laboratory. Multiple size classes were sampled to avoid  
195 sampling single cohorts (potentially related individuals), which might lead to  
196 ambiguous estimates of gene flow and genetic structure. A total of 30 samples per site  
197 were used for genetic analysis. Additional samples from the Victorian Central and  
198 Eastern Zone fisheries were also included to gain insights into the broader spatial  
199 patterns of genetic connectivity (collected in 2012). Field collections and biopsies  
200 were performed following the procedures described in the previous paragraph.

201

202 *LIDAR informed localized spatial sampling*

203 Bathymetric LiDAR data for the entire Victorian coastline were acquired by Fugro  
204 LADS Corporation Pty Ltd., in 2007, in partnership with the Victorian State  
205 Government (Department of Sustainability and Environment). All LiDAR data were  
206 collected using a LADS Mk II acquisition system coupled with a GEC-Marconi  
207 FIN3110 internal motion-sensing system and a dual frequency kinematic GPS, which  
208 was mounted to a DeHavilland Dash-8 aircraft using a fixed-wing platform. The flight  
209 lines were spaced at  $\approx 220$  m apart with an acquisition swath width of 240 m, leaving  
210 a swath overlap of  $\approx 10$  m. The bathymetric LiDAR consisted of two laser scanners:  
211 near infrared laser (at 1064 nm) reflected at the water surface is used for the collection  
212 of topographical data, and the green laser (at 532 nm), to capture the reflectance of the  
213 laser light from the seabed. A portion of the reflected light is collected by the  
214 receivers and the time elapsed between two eco pulses and the speed of light in the  
215 water determines the depth. After the processing of primary data, the resulting digital  
216 elevation model (DEM) provided a continuous representation of the seabed surface in  
217 grid format at an optimal spatial resolution of 5 m and maximum depth of 37 m. This  
218 data layer was used to infer the spatial extent and connectedness of reef habitats  
219 reliably out to a depth of 25 m for the entire Victorian coastline.

220 Replicated hierarchical sampling at fine spatial scales was conducted to assess  
221 the extent of larval recruitment among Western Zone reef patches, and to test the  
222 hypothesis that abalone larval movement is limited ( $<100$  m), with stocks being  
223 largely self-recruiting units ([McShane et al. 1988](#); [Prince et al. 1987, 1988](#)). Under a  
224 local recruitment model, a significant correlation between relatedness and geographic  
225 distance is expected ([Miller et al. 2014](#); [Palumbi 2003](#)). Using LiDAR habitat  
226 mapping ([Jalali et al. 2015](#)), a variety of isolated, continuous, exposed and protected  
227 reef structures with historically variable fishing productivities were identified from  
228 the Killarney area in the Western Zone fishery (Appendix 2; [Zavalas et al. 2014](#)).

229 Abalone specimens were subsequently sampled across a range of spatial scales  
230 (0–6.6 km), with 10 individuals being collected within a 10 m radius from 16  
231 randomly distributed sites representing six reef structures (Figure 2a; Appendix 2).  
232 Some of these reefs were only 10s to hundreds of meters square, where population  
233 densities were very limited and extensive searching was required to find any abalone.  
234 Divers informed us these sites were seldom visited due to historically low  
235 abundances. Thus aggregations on these reefs were likely to comprise less than

236 several hundred individuals. On more extensive commercially productive areas of  
237 reef, an immediate post-disease survey at Killarney during late 2007 found 65 patches  
238 of blacklip abalone with a median of two abalone per patch at 3.1 m spacing between  
239 patches within an area of 320 m<sup>2</sup> (Gorfine et al. 2008). A follow-up survey conducted  
240 during early 2010 at the same sites using the same method found 62 patches within  
241 the same area with a median of three abalone per patch (range 1–21) spaced at 4.0 m  
242 intervals (range 0.3–55.8 m) between patches. Assuming local populations can be  
243 defined by abalone within a radius of about 100m as suggested by Prince *et al.* (1987,  
244 1988), this would indicate a median local population size of about 4680 adults (60%  
245 of the observable population), with one quarter of the sub-populations (inter-breeding  
246 aggregations) having no more than 720 mature individuals, consistent with much  
247 smaller effective population sizes. Assuming there is little migration from non-local  
248 sources (McShane *et al.* 1988; Prince *et al.* 1987, 1988), we expect local populations  
249 of this size to be at migration-drift equilibrium. Theoretically, the time needed for  
250 populations to reach equilibrium is equal to the sum of effective population size ( $N_e$ )  
251 multiplied by generation time (Whitlock & McCauley 1999). The generation time of  
252 *H. rubra* is approximately four years (Andrew 1999), therefore equilibrium should be  
253 reached within several hundred to a few thousand years (<4000). This is well within  
254 the expected timing of post-glacial habitat colonisation (12,000 years; (Hewitt 2004).  
255 A 50 mg non-lethal tissue biopsy was collected from each of the 160 live specimens  
256 following the previously described protocol, and the specimens were returned to the  
257 point of collection. This procedure was repeated at two additional locations from the  
258 Eastern Zone fishery for comparative purposes, with eight sites distributed across six  
259 reef complexes being targeted in the Marlo area (spatial scales 0–3.5 km; Figure 2b),  
260 and four sites representing two reef complexes at Petrel Point (0–5.0 km; Figure 2c).

#### 261 262 *DNA extraction*

263 Approximately 10 mg of muscle tissue from each *H. rubra* specimen was used for  
264 DNA extraction. Total genomic DNA was extracted using a modified Chelex®  
265 extraction protocol (Walsh *et al.* 1991). Individual samples were macerated using a  
266 sterile scalpel blade and placed into separate 0.5 ml microcentrifuge tubes, along with  
267 3 µL of proteinase K and 200 µL of 5% Chelex® solution. Samples were  
268 subsequently digested at 55 °C for 60 min, followed by a final incubation at 95 °C for  
269 15 min with periodic vortexing. Extractions were stored at -20 °C until required. Prior

270 to Polymerase Chain Reaction (PCR) amplification, extractions were spun at 13,000  
271 rpm for 2 min. Aliquots from the bottom half of the supernatant immediately above  
272 the Chelex ® resin was used for PCR amplification.

273

#### 274 *Microsatellite genotyping*

275 We took particular care with our selection of microsatellite markers, as null alleles at  
276 microsatellite loci are common in marine molluscs including *H. rubra* ([Appleyard et al. 2009](#);  
277 [Astanei et al. 2005](#); [Brownlow et al. 2008](#); [Conod et al. 2002](#); [Huang et al. 2000](#);  
278 [Miller et al. 2009](#); [Temby et al. 2007](#)). Null alleles may bias estimates of  
279 genetic structure ([Brownlow et al. 2008](#); [Hedgecock et al. 2004](#); [Lemer et al. 2011](#);  
280 [Miller et al. 2013](#)). To avoid this potential source of bias, we assessed the  
281 performance of 27 microsatellite loci developed by Evans *et al.* ([2000](#)) and Baranski  
282 *et al.* ([2006](#)), some of which have been previously used for population genetic studies.  
283 This procedure was achieved by genotyping 30 individuals from two sample sites  
284 (Whites Beach and Bridgewater; Appendix 1 site codes BW & W) at each of the 27  
285 loci, and measuring their respective conformity to Hardy-Weinberg (HW)  
286 expectations. Those loci that differed significantly from HW expectations were  
287 excluded from further analysis. A total of 15 polymorphic microsatellite loci were  
288 selected for subsequent population genetic analysis of *H. rubra* (Appendix 3). These  
289 loci were amplified by multiplex PCR using Eppendorf Mastercycler gradient units  
290 following the protocol described by Blacket *et al.* ([2012](#)). Microsatellite profiles were  
291 examined and scored manually using GeneMapper version 4.0 (Applied Biosystems).

292

#### 293 *Reduced representation genome library preparation*

294 Genomic analyses were conducted on samples of 10 *H. rubra* individuals from 9  
295 Western Zone sites (90 individuals in total). Sample sites included all sites from the  
296 ‘broad geographic sampling’ microsatellite analysis (Figure 1; Appendix 1), plus an  
297 additional site from the ‘localized spatial sampling’ analysis from the Killarney region  
298 (site number 1; Figure 2A, Appendix 2). Genomic DNA was extracted from 10 mg of  
299 muscle tissue with Qiagen DNA Blood and Tissue kits (Venlo, Limburg, NL), and  
300 reduced representation genome libraries were prepared with a modified Genotyping By  
301 Sequencing (GBS) protocol of Elshire *et al.* ([2011](#)). 500 ng of genomic DNA from  
302 each individual was digested in a 20 uL reaction containing 4 units of MspI for 2  
303 hours at 37 °C, without a heat kill step. Digestion products were then ligated to

304 modified P1 and P2 adapters with 80 unique barcode combinations to allow for  
305 subsequent multiplexing of all individuals. 50 uL ligations were performed containing  
306 the MspI digested DNA, 1.125 ng of P1 and P2 adapters, 400 units of T4 ligase and  
307 1× T4 buffer (New England Biolabs, Beverly MA, USA). Ligations were incubated at  
308 16 °C for 90 minutes followed by a 30 min denaturation at 80 °C. Adapter-ligated  
309 DNA fragments were purified using a Qiagen MinElute® PCR purification kit  
310 (Redwood City, CA, USA), eluted in 20 ul of ddH<sub>2</sub>O, and subsequently used for PCR  
311 amplification. 50 uL PCRs were performed using 2 x MyTaq™ HS Mix (Bioline,  
312 Taunton, MA, USA) and containing 0.2 uM each of Illumina Dual Index Sequencing  
313 Primers 1 & 2 (Illumina Inc., San Diego, CA, USA) and 10 uL of above purified  
314 DNA. PCR conditions were: 95 °C for 1 min, 24 cycles of 95 °C for 30 s, 65 °C for 30  
315 s, 72 °C for 30 s, and a final extension step of 72 °C for 5 min. DNA quantitation and  
316 qualitative analysis of individual PCR products were performed on a MCE®-202  
317 MultiNA with a DNA-1000 kit (Shimadzu, Kyoto). Samples were then pooled  
318 equimolar, and library amplicons between 250-600 bp were extracted from an agarose  
319 gel prior to sequencing on a single HiSeq™ 2000 (Illumina, San Diego) lane at the  
320 Australian National University Biomolecular Resource Facility (Australian Capital  
321 Territory, Australia).

322

### 323 *Bioinformatics processing and genotyping*

324 Sequences were first processed using the FASTX-Toolkit program  
325 ([http://hannonlab.cshl.edu/fastx\\_toolkit/](http://hannonlab.cshl.edu/fastx_toolkit/)) by trimming the raw reads to 80 bp length  
326 and discarding all reads that had a Phred score below 20. We used the *de novo*  
327 program from *Stacks* 1.19 ([Catchen et al. 2013](#)) to create a catalogue of SNPs and  
328 genotypes for all individuals. Because we expected a high level of intrapopulation  
329 genetic diversity [based on microsatellite data from this and previous studies; ([Miller](#)  
330 [et al. 2009](#); [Miller et al. 2014](#))], we tested several combinations of parameters that  
331 allowed *de novo* assembly of GBS loci from orthologous sequences while rejecting  
332 paralogous sequences. The following parameter ranges included: the maximum  
333 distance allowed between stacks ( $M = 3$  and  $7$ ), the distance between loci in the  
334 catalogue ( $n = 0$  and  $3$ ), while the minimum depth of coverage to form a stack was  
335 kept constant ( $m = 3$ ). SNPs and genotypes at each locus were called using a  
336 maximum likelihood framework with default *Stacks* 1.19 parameters at the  
337 significance level of 5%.

338

339 *Microsatellite data analyses*

340 *Broad geographic sampling*

341 Descriptive statistics for the microsatellite data were obtained using FSTAT, version  
342 2.9.3 ([Goudet 1995](#)), including allelic richness per population averaged over loci,  
343 Weir and Cockerham's measure of  $F_{IS}$ , locus-by-locus and global estimates of  $F_{ST}$   
344 (with 95% confidence limits) ([Weir & Cockerham 1984](#)) population pair-wise  
345 measures of  $F_{ST}$  and their significance determined using permutations (10,000), and  
346 pairs of loci tested for linkage disequilibrium using a log-likelihood ratio test. To  
347 overcome potential limitations of  $F_{ST}$  due to high amounts of within-population  
348 genetic variation, we also calculated a standardized measure of  $F'_{ST}$  according to  
349 Hedrick ([2005](#)) and Meirmans ([Meirmans 2006](#)). The software MICRO-CHECKER  
350 ([Van Oosterhout et al. 2004](#)) was used to assess microsatellite loci for null alleles and  
351 for scoring errors. The frequency of null alleles per locus was obtained using the  
352 'Brookfield 1' formula, as evidence of null homozygotes across loci was not observed  
353 ([Brookfield 1996](#)). The sequential Bonferroni procedure ([Rice 1989](#)) was used to  
354 adjust significance levels when performing multiple simultaneous comparisons.

355 Estimates of observed ( $H_O$ ) and expected ( $H_E$ ) heterozygosity were  
356 determined using the Excel Microsatellite Toolkit ([Park 2001](#)) and deviations from  
357 HW equilibrium were tested using Genepop version 3.4 ([Raymond & Rousset 1995](#)).  
358 An analysis of molecular variation (AMOVA) was performed in GenAIEx version  
359 6.501 ([Peakall & Smouse 2006](#)) based on pairwise  $F_{ST}$  as the distance measure, with  
360 10,000 permutations and missing data for loci set at 10%. We partitioned variation  
361 among regions (Victorian fishing Zones;  $F_{RT}$ ), among locations within regions ( $F_{LR}$ ),  
362 and within locations ( $F_{WL}$ ). Regressions and Mantel tests of Slatkin's linearized  $F_{ST}$   
363 transformation [ $F_{ST}/(1 - F_{ST})$ ] ([Rousset 1997](#)) with the natural log of geographical  
364 distance were calculated using GenAIEx ([Peakall & Smouse 2006](#)). The significance  
365 of Mantel tests was determined by permutation (10,000 randomisations). A factorial  
366 correspondence analysis (FCA), implemented in GENETIX version 4.05 ([Belkhir et](#)  
367 [al. 2004](#)), was used to summarise patterns of genetic differentiation between  
368 individuals across sample sites. The first two underlying factors that explain the  
369 majority of variation in multi locus genotypes across loci were plotted.

370 Independent analyses of population structure were conducted using Bayesian  
371 analyses. Based solely on genetic data, STRUCTURE ([Pritchard et al. 2000](#))

372 identifies the number of distinct clusters / populations, assigns individuals to clusters  
373 and identifies migrants and admixed individuals. To determine the number of  
374 populations ( $K$ ), five independent simulations for  $K = 1-16$ , with 100,000 burn-ins  
375 and 1,000,000 data iterations were run. Analysis was performed using the admixture  
376 model of population structure (i.e. each individual draws some fraction of their  
377 genome from each of the  $K$  populations) and allele frequencies correlated among  
378 populations (i.e. allele frequencies in the different populations are likely to be similar,  
379 due to factors such as migration or shared ancestry). The most likely  $K$  was estimated  
380 using Evanno's  $\Delta K$  (Evanno *et al.* 2005), which was implemented in Structure  
381 Harvester (Earl & Vonholdt 2012).

382

### 383 Localized spatial sampling

384 For each of the fine scale sampling sites, estimates of global  $F_{ST}$  and  $F'_{ST}$  were  
385 determined using the methods outlined above. An analysis of molecular variation  
386 (AMOVA) was also run to partition variation among reef patches ( $F_{RT}$ ), and among  
387 locations within patches ( $F_{LR}$ ). Spatial autocorrelation analysis was performed in  
388 GenAlEx 6.501 to assess the spatial genetic structure of *H. rubra* at fine spatial scales  
389 among the Killarney, Marlo and Petrel Point sampling replicates, providing a test of  
390 localised stock recruitment. Distance classes for this analysis were based on the 'equal  
391 sample size' option, and with 9999 permutations to test for levels of significance.  
392 Spatial autocorrelation analysis was also replicated with SPAGeDi 1.2 (Hardy &  
393 Vekemans 2002). We estimated the Queller and Goodnight (1989) relatedness  
394 coefficient among pairs of individuals belonging to the same *a priori* defined distance  
395 classes. For each class, random permutations in the spatial locations of individuals  
396 (10000 permutations) were then used to assess deviations of the relatedness  
397 coefficient  $R$  from 0. Distance classes were chosen so that they contained more than  
398 100 pairwise comparisons, had a participation index >50% and a coefficient of  
399 variation of participation of less than 1 (Hardy & Vekemans 2002). Deviation from 0  
400 means that individuals within a given distance class are significantly more (positive  
401 values) or less (negative values) related than random. To assess the reliability of the  
402 results obtained with the Queller and Goodnight (1989) relatedness coefficient, we  
403 repeated the analyses using two other relatedness estimators; namely, Lynch and  
404 Ritland's (1999) relatedness coefficient ( $r$ ) and the kinship coefficient of Loiselle *et*  
405 *al.* (1995). To determine the frequency and direction of larval dispersal between

406 collection sites, assignment tests were conducted for the Killarney, Marlo and Petrel  
407 Point sampling sites using the 'leave one out' setting implemented in GenAIEx 6.501.

408

#### 409 *Genome-wide SNP data analyses*

410 We performed analyses of population structure with a subset of polymorphic GBS  
411 loci found in at least 60% of individuals at each sampling location (Boehm *et al.*  
412 2015; Dierickx *et al.* 2015) and having SNPs with a global minor allele frequency of  
413  $\geq 0.01$ . Estimates of genetic diversity, observed ( $H_O$ ) and expected ( $H_E$ )  
414 heterozygosity across loci, marker independence, as well as global and pair-wise  
415 measures of  $F_{ST}$  (Weir and Cockerham 1984), were calculated using Genepop.  
416 Population genetic differentiation was also assessed using the Discriminant Analysis  
417 of Principal Components (DAPC) implemented in the *adegenet* package for R  
418 ([Jombart 2008](#); [Jombart & Ahmed 2011](#)).

419 We applied a Bayesian simulation-based test implemented in the software  
420 BayeScan 2.1 ([Foll & Gaggiotti 2008](#)) to identify candidate loci putatively under  
421 selection (outlier loci). The test directly estimates a posterior probability for each  
422 locus and a reversible-jump MCMC approach to selection. Representing an extension  
423 of a test proposed by Beaumont & Balding ([2004](#)), BayeScan estimates population-  
424 specific  $F_{ST}$  coefficients, which integrate population-specific and locus-specific  
425 (selection) effects. Preliminary tests were conducted using a burn-in of 10,000  
426 iterations, a thinning interval of 50 and prior odds of 1,000 ([Foll & Gaggiotti 2008](#)).  
427 Four independent runs were performed for each of the two data sets to account for the  
428 consistency of the detected outliers. The loci were ranked according to their estimated  
429 posterior probability and all loci with a value over 0.990 were retained as outliers.  
430 This corresponds to a  $\log_{10}$  Bayes factor of more than 2, making it possible to be  
431 confident in the validity of the model. Repeated analyses showed that this was  
432 sufficient to achieve convergence.

433

#### 434 *Population genetic simulations*

435 To infer the likely numbers of effective migrants per generation required to drive the  
436 observed patterns of genetic differentiation in *H. rubra* in Victorian waters, we ran  
437 individual-based forward-time population genetic simulations in simuPOP (Peng and  
438 Kimmell 2005) to infer the likely numbers of effective migrants per generation  
439 required to drive the observed patterns of genetic differentiation in *H. rubra* in

440 Victorian waters. We were particularly interested in determining the potential level of  
441 migration needed to drive the observed pattern of panmixia within the Western and  
442 Central Zone fisheries (excluding the Port Phillip Bay site). The most important  
443 assumptions of the model are: fixed number of populations ( $n = 10$ ) of equal size ( $N_e$   
444  $= 100, 500, 1000$  or  $10,000$ ) that interact through migration of globally dispersing  
445 migrants at a certain rate  $m$  (proportion of migrants relative to population size). We  
446 chose a wide range of effective population size estimates given the hierarchical nature  
447 of our sampling design (broad and local scale sampling) which included reef systems  
448 with high and low density populations. Simulations were run with the following  
449 number of migration rates per generation: 0.1, 1.0, 5.0, and 10.0%. Each migrant's  
450 destination was assigned randomly according to the probability of migration into each  
451 subpopulation ( $m/(n - 1)$ ). Neutral step-wise mutations also occurred at a rate  $\mu$  of  $10^{-4}$   
452 per microsatellite locus per generation. We considered the  $n$ -island migration model  
453 only, because we did not find empirical support for the stepping-stone model (lack of  
454 isolation-by-distance in the region, see Results). Genotypes were initialized with  
455 observed (empirical) allele frequencies at 10 independent microsatellite loci,  
456 assuming random mating. Simulations were run for 1000 generations with 100  
457 replicates. Global  $F_{ST}$  (Weir and Cockerham 1984) was calculated for a random  
458 sample of 30 individuals drawn from each subpopulation every ten generations and  
459 was averaged over 100 replicate runs.

460

#### 461 *Statistical power to detect genetic structure*

462 POWSIM version 4.0 (Ryman & Palm, 2006) was used for evaluation of the alpha  
463 error and statistical power of the microsatellite and SNP loci for accurately detecting  
464 different levels of  $F_{ST}$ . This takes into account the sample sizes, number of loci and  
465 average allele frequencies from the respective datasets. POWSIM is limited to a  
466 maximum of 50 loci, therefore the statistical power of the SNP dataset was estimated  
467 from 10 replicate analyses based on 50 randomly selected SNP loci.

468

## 469 **Results**

### 470 *Microsatellite data analyses*

#### 471 Broad geographic sampling

472 A total of 445 *H. rubra* specimens from 14 sampling sites were genotyped at 15  
473 microsatellite loci. Marker independence was confirmed across all sampling sites,

474 with linkage disequilibrium analyses indicating no significant linkage between loci;  
475 however, MICROCHECKER analyses indicated the potential influence of null-alleles  
476 at loci Hrub10.B11a, Hrub1.H07, Hrub6.CO4, Hrub12.E10 and Hrub9.B05 with  
477 evidence of an excess of homozygotes. Departures from HW equilibrium at these loci  
478 could also be potentially driven by additional factors other than null alleles, such as  
479 selection. All subsequent analyses were conducted including and excluding these loci.  
480 Each analysis was congruent, indicating that the exclusion of these loci had no  
481 significant effect on the overall patterns found. Nonetheless, we present and discuss  
482 the results based on the analyses of the 10 reliable loci only. A total of 138 alleles  
483 were detected, with a mean of 8.17 alleles per locus at all sites. Allelic richness across  
484 all loci and sites ranged from 6.28 to 7.53 (Table 1), while expected heterozygosities  
485 ranged from 0.66 to 0.73 (mean  $H_E = 0.70$ ). Significant ( $P < 0.05$ ) departures from  
486 HW equilibrium were observed at two sampling sites (Table 1), each also  
487 accompanied by significant and positive  $F_{IS}$  values, indicating a deficit of  
488 heterozygotes. These estimates were influenced by single loci only and differed  
489 between sites.

490 Global estimates of  $F_{ST}$  and  $F'_{ST}$  were significantly different from zero ( $F_{ST} =$   
491  $0.010$ ,  $P < 0.01$ ;  $F'_{ST} = 0.027$ ,  $P < 0.01$ ) indicating weak genetic structure amongst  
492 sampling locations (Table 2). Pairwise estimates of  $F_{ST}$  and  $F'_{ST}$  indicate that this  
493 result is largely driven by genetic differentiation among sites separated by Wilsons  
494 Promontory in Bass Strait (Table 3), a common biogeographic barrier for many  
495 marine species (Miller *et al.* 2013). In addition, weak genetic differentiation was  
496 observed involving the Port Phillip Bay sample location (PP), a semi-isolated  
497 population not exposed to the open southern ocean. Excluding the samples from the  
498 Eastern Zone fishery sites and Port Phillip Bay,  $F_{ST}$  and  $F'_{ST}$  were not significant  
499 ( $F_{ST} = 0.003$ ,  $P > 0.05$ ;  $F'_{ST} = 0.009$ ,  $P > 0.05$ ), indicating a lack of genetic structure  
500 across the Western and Central Zone fisheries. These findings are supported by the  
501 observed pairwise  $F_{ST}$  and  $F'_{ST}$  estimates across these sites which were weak and  
502 generally non-significant (only five of 91 pairwise comparisons were significant).

503 AMOVA analyses indicated limited genetic variation among sites. The  
504 majority of variation across microsatellite loci was explained by variation within sites  
505 (99%;  $P < 0.001$ ), while between site variation was 1% ( $P < 0.001$ ). Hierarchical  $F$ -  
506 statistic analysis showed weak but significant differentiation between sample location  
507 within and between regions ( $F_{LR}$  and  $F_{RT} = 0.005$ ,  $P < 0.001$ ; Table 2), but differences

508 were no longer significant following the exclusion of the Eastern Zone fishery and  
509 Port Phillip Bay samples ( $F_{LR}$  and  $F_{RT} = 0.002$ ,  $P > 0.05$ ). FCA also indicated limited  
510 genetic structure, with the first two factors representing just 4.05% of the total  
511 variation between samples (2.24% and 1.88%, respectively). Appendix 5 highlights  
512 the low differentiation between individuals from the Victorian sample sites.

513 Mantel tests showed a moderately strong relationship between Slatkin's  
514 linearized  $F_{ST}$  and the natural log of geographic distance (Mantel  $r = 0.45$ ,  $P < 0.01$ ).  
515 Regression analysis showed this relationship to be positive and linear ( $R^2 = 0.20$ ,  $P <$   
516  $0.01$ ). This relationship is no longer significant once the Eastern Zone fishery sites are  
517 removed from the analysis (Mantel  $r = 0.25$ ,  $P > 0.05$ ; regression  $R^2 = 0.07$ ,  $P > 0.05$ )  
518 suggesting high levels of gene flow across the Western and Central Zone fisheries.

519 Bayesian analysis using STRUCTURE identified a single cluster ( $K = 1$ ),  
520 reflecting a lack of genetic structure across the study area. Although convergence  
521 initially indicated two clusters, this clearly represented a sub-optimal plateau, as the  
522 admixture histogram for  $K = 2$  showed all individuals sharing an equal proportion of  
523 mixed ancestry from each of the two putative clusters. A limitation of the Evanno  
524 method for estimating the most likely number of population clusters is that  $\Delta K$  cannot  
525 be calculated for a single population cluster ([Evanno et al. 2005](#)). This leads to an  
526 artifactual convergence to the 'next best'  $K$  (in this case  $K = 2$ ). Inspection of the log-  
527 likelihood plots confirmed that the highest value was indeed for  $K = 1$ .

528

### 529 *Localized spatial sampling*

530 Global estimates of  $F_{ST}$  and  $F'_{ST}$  were not significant ( $P > 0.05$ ) for any of the three  
531 fine scale sampling locations (Killarney:  $F_{ST} = 0.001$ ,  $F'_{ST} = 0.000$ ; Marlo:  $F_{ST} =$   
532  $0.009$ ,  $F'_{ST} = 0.017$ ; Petrel Point:  $F_{ST} = 0.006$ ,  $F'_{ST} = 0.003$ ; Table 2) indicating no  
533 genetic structuring amongst sites within regions. Hierarchical AMOVA showed most  
534 variation is present at the reef scale (Killarney = 100%; Marlo and Petrel Point =  
535 99%;  $P < 0.01$ ), with analysis of  $F$ -statistics generally showing no significant  
536 differentiation among reef patches or among sites within reef patches (Killarney:  $F_{RT}$   
537  $= 0.002$ ,  $F_{LR} = 0.000$ ,  $P > 0.05$ ; Marlo:  $F_{RT} = 0.011$ ,  $P < 0.05$ ;  $F_{LR} = 0.000$ ,  $P > 0.05$ ;  
538 Petrel Point:  $F_{RT} = 0.000$ ,  $F_{LR} = 0.009$ ,  $P > 0.05$ ; Table 2). These findings are further  
539 supported by Bayesian analyses with STRUCTURE, which indicated a single  
540 population cluster ( $K = 1$ ) based on the inspection of the log-likelihood scores  
541 ([Evanno et al. 2005](#)).

542 A spatial autocorrelation analysis was performed using the multilocus  
543 genotypes from 149 *H. rubra* specimens from 16 sites and six reef complexes from  
544 the Western Zone Killarney area. The relatedness coefficient ( $R$ ) was calculated for  
545 all pairs of individuals, involving 11,036 pairwise comparisons across 11 distance  
546 classes, ranging from 0 to 6,600 m. Identical independent analyses were also  
547 conducted in the Eastern Zone for 80 individuals from Marlo (3,160 pairwise  
548 comparisons across eight distance classes, ranging from 0 to 3,500 m) and 40  
549 individuals from Petrel Point (741 pairwise comparisons across seven distance  
550 classes, ranging from 0 to 5,000 m). All analyses using the different relatedness  
551 measures indicated no significant patterns between relatedness and geographic  
552 distance at any of the scales (Figure 3). Estimates of  $R$  were not significantly greater  
553 at reef scales, or at small geographic distances, reflecting a complete absence of local  
554 genetic structure. In all cases, similar results were obtained; therefore, we only present  
555 those derived from GenAlEx analyses.

556 Assignment tests suggest frequent larval migration between reef patches for  
557 the Killarney, Marlo and Petrel Point sampling locations. Only 7%, 13% and 38%  
558 (respectively) of individual multilocus genotypes were assigned to their collection  
559 locations, indicating high levels of migration amongst reef patches in each region of  
560 the fishery. Geographic patterns of assignment in each region are absent, suggesting  
561 larval dispersal is haphazard. This is consistent with the above analyses suggesting no  
562 relationship between genetic and geographic distance.

563

#### 564 *Population genomic analysis*

565 Variation of *de novo* assembly parameters ( $M$ ,  $n$ ) had little effect on estimates of  
566 population genetic differentiation. The total number of GBS tags varied between  
567 2,310,175 and 3,694,442, and the number of polymorphic tags ranged from 790,938  
568 and 976,894 (Table 4). However, due to low overall depth of coverage, the number of  
569 tags shared between at least 60% of individuals within and across the eight Western  
570 Zone sites ranged from only 641 to 1,121 and a total number of SNPs based on a  
571 global minor allele frequency of 10% ranging from 1,425 to 1,763 (Table 4).  
572 BayeScan analyses failed to identify any loci that are likely to be influenced by  
573 selection and marker independence was confirmed across all sample sites with linkage  
574 disequilibrium analyses indicating no significant linkage between loci. Across loci  
575 each site met HW expectations ( $P > 0.05$ ), although some significant departures were

576 observed at the locus level ( $P < 0.05$ ). These were infrequent and differed across sites,  
577 justifying the inclusion of all loci for analyses of population differentiation. Despite  
578 the difference in the number of assembled tags and SNPs across, the overall estimates  
579 of heterozygosity and  $F_{IS}$  were highly consistent between the datasets created with  
580 different parameter combinations. In each case a weak yet significant excess of  
581 heterozygotes was evident across loci and sites ( $H_O = 0.21 - 0.25$ ,  $H_E = 0.18 - 0.20$ ;  
582  $0.25$ ;  $F_{IS} = -0.18-0.28$ ; Appendix 7), likely reflecting high levels of genetic diversity  
583 and outcrossing. Importantly, there was no evidence of significant population  
584 differentiation regardless of parameter settings (global  $F_{ST} = 0.001-0.005$ ,  $P > 0.05$ ,  
585 pairwise  $F_{ST}$  found in Table 4 and Appendix 4), further supported by DAPC whereby  
586 any notable genetic structure among the Western Zone study sites was absent  
587 (Appendix 6).

588 It is important to note that results from the SNP dataset alone should be  
589 interpreted with some caution, given very limited sample sizes. However, these  
590 inferences are concordant with those from the microsatellite dataset, and collectively  
591 these indicate a lack of genetic structure within the Western Zone abalone fishery.

592

#### 593 *Population genetic simulations*

594 The effect of various levels of gene flow on the observed genetic structuring across  
595 the Western and Central Zone *H. rubra* fisheries was deduced from the simulation  
596 results (Figure 4). Based on assumed effective population sizes of 500 to 1000  
597 individuals, a migration rate of 5 to 10% per generation is needed to achieve an  $F_{ST}$   
598 equal to the observed global  $F_{ST}$  of 0.003. For a proportion of the sites we sampled at  
599 fine scales the estimated local population sizes would have been very small with less  
600 than several hundred individuals, therefore migration rate considerably greater than  
601 10% is required to suppress significant genetic differentiation. When the assumed  
602 effective population size is  $\geq 10,000$  this level of population differentiation can be  
603 potentially driven by a migration rate as low as  $<1\%$  per generation.

604

#### 605 *Statistical power to detect genetic structure*

606 Power analyses indicated that the microsatellite and SNP marker datasets are both  
607 capable of detecting a true  $F_{ST}$  of 0.01 or larger with a probability of 99% or more,  
608 and an  $F_{ST}$  as low as 0.005 with 95% confidence. The alpha error for both datasets  
609 (i.e. the probability of obtaining false significances when the true  $F_{ST} = 0$ ) was zero.

610 As only 50 SNP loci were tested for each replicate analysis, the SNP genomic datasets  
611 (ranging from 1,425 to 1,763 SNPs in total) are expected to be substantially more  
612 powerful than the 10 microsatellites included in this study when detecting spatial  
613 patterns of genetic structure and gene flow.

614

## 615 **Discussion**

### 616 *Spatial patterns of genetic connectivity*

617 Using *H. rubra* as a model system, we demonstrate how genotyping methods can be  
618 combined and integrated with high-resolution bathymetric data to overcome current  
619 limitations of determining genetic structure and population connectivity in marine  
620 benthic species. As in previous population genetic studies of *H. rubra* (Brown 1991;  
621 Conod et al. 2002; Li et al. 2006; Miller et al., 2009), we found that larval movement  
622 may homogenise gene frequencies across broad geographical distances, in this case  
623 ~1400 km of the Victorian coastline. However, these findings do not provide a  
624 reliable estimate of population connectivity, as panmixia can be theoretically achieved  
625 by low levels of effective migrants per generation ([Lowe & Allendorf](#)  
626 [2010](#); [Meirmans & Hedrick 2011](#); [Waples et al. 2008](#)). This is evident from our  
627 simulations. Also, like most previous studies, our broad geographic sampling was  
628 biased towards commercially viable stocks with large effective sizes ( $N_e > 10,000$ )  
629 and potentially not at migration-drift equilibrium (Hauser & Carvalho 2008; Slatkin  
630 1993).

631 Based on high-resolution bathymetric data of the Victorian coastline we  
632 sampled within and across abalone habitats at a range of spatial scales, and targeted  
633 small local populations expected to be susceptible to drift processes (assuming local  
634 populations are predominantly self-recruiting; ([McShane et al. 1988](#); [Prince et al.](#)  
635 [1987, 1988](#)). In contrast to results obtained with Tasmanian fisheries where there  
636 appears to be fine scale (hundreds of meters) genetic structure with limited gene flow  
637 among adjacent populations (Miller et al. 2009), we found no genetic structuring at  
638 local spatial scales, suggesting recruitment success is not predominantly dependent on  
639 local reef sources. The local spatial sampling undertaken in this study included reef  
640 complexes in the Killarney area of the Western Zone fishery with small abalone  
641 populations (tens to hundreds of individuals) and in areas protected from wave  
642 exposure and major ocean currents. This provided a suitable experimental design to  
643 test for local genetic structure and for dispersal through relatedness analyses. These

644 analytical methods applied have been used previously to identify significant patterns  
645 of local genetic structure in various benthic marine species from South Eastern  
646 Australia, including *Haliotis* species ([Miller et al. 2014](#); [Piggott et al. 2008](#); [Teske et](#)  
647 [al. 2015a](#); [Teske et al. 2015b](#)). In this case the absence of local genetic structure and  
648 relatedness at the reef scale points to substantial distant recruitment. Our simulations  
649 suggest  $m$  needs to be considerably greater than 10% of the effective population to  
650 suppress genetic differentiation (e.g. Killarney  $F_{ST} = 0.001$ ,  $P > 0.05$ ) within these  
651 relatively small populations. Larval supply is likely to be markedly greater than  $m$ ,  
652 because larval and juvenile mortality rates are high in abalone species including *H.*  
653 *rubra* (Day & Leorke 1986; McShane 1991; Shepherd & Breen 1992; Takami et al.  
654 2008).

655 Palumbi (2003) suggested that the genetic signal of isolation by distance is  
656 relatively robust in a wide variety of marine species and populations. Even marine  
657 species with high dispersal are expected to show isolation by distance, which  
658 therefore provides a measure of dispersal distance limitation. While under a large  
659 population model sampling error associated with estimating  $F_{ST}$  may be higher than  
660 expected levels of genetic differentiation (Waples 1998), we found no relationship  
661 between genetic and geographic distance which points to high larval dispersal and  
662 panmixia. Our spatial autocorrelation and assignment test results differ from those for  
663 greenlip abalone, *Haliotis laevigata*, in South Australian waters; Miller et al. (2014)  
664 found significant spatial autocorrelation and confident assignment of 97 to 100% of  
665 adult genotypes back to the collection locale, indicating high levels of self-  
666 recruitment. In contrast we found no significant spatial autocorrelation and lower  
667 assignment estimates across all Western and Eastern Zone localized spatial sampling  
668 locations (as low as 7%), reflecting higher levels of connectivity for *H. rubra* in  
669 Victorian waters. Collectively this suggests that abalone stocks from the Western and  
670 Central Zone fisheries (South Australian border to Wilsons Promontory), and stocks  
671 within the Eastern Zone fishery (east of Wilsons Promontory) represent well  
672 connected panmictic units, with high levels of gene flow within and between reef  
673 patches (at least up to 6,600 m separation). Larval supply and recruitment success  
674 may therefore not depend predominantly on local reef sources.

675 We did observe some genetic structuring between abalone stocks occurring  
676 west (Western and Central Zone fisheries) and east (Eastern Zone fishery) of Wilsons  
677 Promontory, indicating limits to gene flow across the wider region. Biogeographical

678 studies suggest that community assemblages either side of the Wilsons Promontory  
679 region differ genetically ([Ayre et al. 2009](#); [Colton & Swearer 2012](#); [Miller et al.](#)  
680 [2013](#); [York et al. 2008](#)). This structuring has been attributed to historical ([Lambeck &](#)  
681 [Chappell 2001](#)) and contemporary physical factors, such as converging ocean  
682 currents, environmental gradients (temperature and salinity), and habitat  
683 discontinuities that persist in the region ([Baines et al. 1983](#); [Colton & Swearer](#)  
684 [2012](#); [Ridgway & Condie 2004](#); [Ridgway & Godfrey 1997](#); Sandery & Kämpf 2007).  
685 Gene flow also appears to be somewhat limited between Port Phillip Bay and the  
686 remaining Central Zone sample locations. Again this is consistent with the  
687 oceanography of the region, with the low flushing rates of Port Phillip Bay  
688 (approximately 270 days; ([Walker 1999](#)) likely limiting gene flow to some extent  
689 between local stocks and those from outside coastal waters.

690

#### 691 *Factors influencing larval movement and recruitment*

692 Our findings, combined with those from previous ecological ([McShane et al.](#)  
693 [1988](#); [Prince et al. 1987, 1988](#)), and genetic ([Miller et al. 2009](#); [Temby et al. 2007](#))  
694 research, indicate that larval movement and recruitment success is likely to be  
695 spatially variable across the *H. rubra* fishery. Population size structure, frequency and  
696 timing of spawning events, the persistence of favourable benthic habitat, and  
697 oceanographic conditions in combination may lead to fluctuating patterns of larval  
698 movement and recruitment success across space and time, as indicated by recent  
699 genetic analyses of pink abalone off the Californian coast ([Coates et al. 2014](#)). Recent  
700 habitat mapping studies have shown that prime blacklip abalone fishing grounds are  
701 found in areas with topographically complex reef systems ([Jalali et al. 2015](#)). Crevice  
702 spaces provide relative shelter from high wave energy for juvenile abalone and  
703 protection from predatory species (Naylor & McShane 2001). Also gutters and surge  
704 channels that dissect the reef surface are thought to concentrate macroalgal drift,  
705 enabling larger blacklip abalone to feed opportunistically whilst aggregated along  
706 vertical reef walls ([Gorfine 2002](#)). Thus the production of larvae and their advection  
707 by currents is likely to vary even within individual reefs. Evidence favouring a  
708 panmictic population with broadscale supply of larvae to contiguous reef systems is  
709 consistent with longshore transport along the Victorian mainland coast. Consequently,  
710 we propose that patterns of larval movement are spatially variable and determined by  
711 factors such as wave exposure and current intensity. Stocks inhabiting protected or

712 semi-protected habitats are perhaps more likely to be locally-recruiting than more  
713 exposed habitats. However our surveys found no direct evidence of this despite  
714 sampling abalone from a complex of exposed and protected isolated reef patches in  
715 the Killarney region.

716 Larval recruitment at the reef scale is also likely to be influenced by ecological  
717 factors, particularly in cases where abalone abundances have been reduced. Hamer *et*  
718 *al.* (2010) found that experimental removals of blacklip abalone resulted in  
719 overgrowth of reef substrate by a range of biota (including colonial invertebrates,  
720 filamentous and foliose algae, and sediment matrix) that appeared to have a negative  
721 influence on recruitment. Similar results of abalone removal were also found by  
722 Strain and Johnston (2012) in Tasmania. However, not all coralline habitats are  
723 dependent on grazing by abalone; instead habitat conditions can be influenced by  
724 additional species groups including gastropods, chitons and urchins (Clarkson &  
725 Shepherd 1985; Day & Branch 2002). Day and Branch (2002) found urchin grazing  
726 maintained encrusting corallines, and abalone larval establishment declined sharply  
727 when urchins were removed and sediment built up over crustose corallines. A  
728 reduction of suitable habitat may therefore contribute to a slow recovery of depleted  
729 local fishing stocks. This is perhaps an alternative interpretation of the results of  
730 Prince *et al.* (1987 & 1988) from which they concluded that there is limited dispersal  
731 of abalone larvae.

732 Estimates of neutral genetic diversity in this study indicate a lack of genetic  
733 structure at the various hierarchical spatial scales, although adaptive genetic diversity  
734 could still be playing a role in the recruitment process. Recent genomic studies of  
735 marine fish and molluscs have demonstrated that under strong selection pressures,  
736 adaptive variation can be maintained in marine environments despite high levels of  
737 gene flow (Galindo *et al.* 2009; Hess *et al.* 2013; Martinez-Fernandez *et al.*  
738 2010; Milano *et al.* 2014; Solas *et al.* 2013). Consequently, patterns of abalone larval  
739 settlement and establishment could be determined by underlying adaptive genetic  
740 variation, where recruitment at the reef scale is dependent on the settlement of  
741 compatible adaptive genotypes. More comprehensive sampling of individuals and  
742 sites across environmental gradients (assisted by high-resolution bathymetric data),  
743 coupled with higher genome coverage and sequencing depth will be needed to assess  
744 adaptive variation.

745

746 *Fisheries management implications*

747 Findings have implications for several areas of management. Importantly, they  
748 provide insights into the risks associated with disease spread, and the likely resistance  
749 of *H. rubra* fishing stocks to environmental disturbance. High levels of stock  
750 connectivity driven by larval movement suggests that any heritable diseases will have  
751 the potential to spread quickly throughout Victorian fisheries. Larval movement will  
752 also facilitate the rapid spread of disease resistance alleles, but the evolution of  
753 resistance may be slow given the large interconnected nature of the Victorian *H.*  
754 *rubra* population. High gene flow and large population sizes are likely to enhance the  
755 resilience of fishing stocks to environmental change generally, as larval supply will  
756 help replenish affected stocks and assist in the maintenance of genetic diversity that  
757 can then respond to natural selection.

758 Stock augmentation activities, such as reseedling and translocation are being  
759 explored as an option for promoting the recovery of depleted Victorian stocks in virus  
760 and urchin affected regions. Genetic risks associated with the translocation of  
761 genotypes across reefs within individual fisheries are expected to be minimal given  
762 evidence of panmixia. However more comprehensive analyses are needed to test for  
763 potential adaptive diversity within *H. rubra* fisheries that might assist in establishing  
764 stock augmentation guidelines ([Weeks et al. 2011](#)).

765 Despite high levels of gene flow within and among Victorian abalone  
766 fisheries, the impact of abalone fishing will still need to be managed at fine spatial  
767 scales to prevent serial depletion ([Karpov et al. 2000](#)), where population densities  
768 may decrease below biological or ecological tipping points to levels from which they  
769 cannot easily recover ([Strain & Johnson 2012](#)). Consequently management strategies  
770 should include conservative minimum size limits and catch controls or caps at  
771 contiguous reef scale. Co-operative Industry initiatives that complement statutory  
772 management by controlling catch at reef scale are also required ([Gilmour et al. 2013](#)).  
773 This will ensure a residual sub-legal sized population on each major reef complex that  
774 is large enough to maintain the area of habitat necessary for sufficient settlement to  
775 replenish population numbers. Nevertheless, the prospect that *H. rubra* populations in  
776 Victoria have a more widespread supply of larvae than previously described should  
777 reassure the government fisheries agency that the spatial scales at which they can  
778 reasonably regulate does not carry as high a risk as previously believed.

779

780 **Conclusion**

781 Genetic studies offer the opportunity to provide insights into genetic structure and  
782 population connectivity at species and communities scales that can assist management  
783 ([Allendorf \*et al.\* 2010](#); [Hauser & Carvalho 2008](#)). The current study demonstrates  
784 how reliable inference of genetic structure in marine benthic systems can be achieved  
785 by using panels of genetic and genomic markers and geospatially informed sampling  
786 strategies. This provides an opportunity to then link genetic data to LIDAR data,  
787 which can in turn assist in the management of ecologically and economically  
788 important benthic species (i.e. rock lobster, scallop, oyster). This also provides an  
789 opportunity to apply advances in the field of ‘landscape genetics’ to the marine  
790 environment to identify the scales that evolutionary processes operate, drivers and  
791 limitations of adaptation to environmental change, and physical seascape features that  
792 influence patterns of genetic structure ([Johansson \*et al.\* 2015](#); [Manel \*et al.\* 2003](#)).  
793 Marine ecosystems are facing increasing threats from resource exploitation,  
794 eutrophication, invasive pests, warming, acidification, disease and habitat destruction  
795 ([Jackson 2010](#)). This is particularly relevant to coastal waters where the proximity to  
796 the world’s major population and industrial centres are located adjacent to these zones  
797 and associated threats. Effective conservation planning, based on genetic studies with  
798 suitably structured sampling regimes, will assist in minimising biodiversity loss and  
799 preserving economic values such as commercial fisheries and ecotourism.

800

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820

## 821 **References**

- 822 Allendorf FW, Hohenlohe PA, Luikart G (2010) Genomics and the future of  
823 conservation genetics. *Nature Reviews Genetics* **11**, 697-709.
- 824 Andrew NL (1999) *Under Southern Seas: The Ecology of Australia's Rocky Reefs*.  
825 University of New South Wales Press, 238 pp.
- 826 Appleyard SA, Carr NA, Elliott NG (2009) Molecular analyses indicate homogenous  
827 structure of abalone across morphologically different *Haliotis rubra*  
828 collections in South Australia. *Journal of Shellfish Research* **28**, 609-616.
- 829 Astanei I, Gosling E, Wilson J, Powell E (2005) Genetic variability and  
830 phylogeography of the invasive zebra mussel, *Dreissena polymorpha* (Pallas).  
831 *Molecular Ecology* **14**, 1655-1666.
- 832 Ayre DJ, Minchinton TE, Perrin C (2009) Does life history predict past and current  
833 connectivity for rocky intertidal invertebrates across a marine biogeographic  
834 barrier? *Molecular Ecology* **18**, 1887-1903.
- 835 Baines PG, Edwards RJ, Fandry CB (1983) Observations of a new baroclinic current  
836 along the western continental slope of Bass Strait. *Australian Journal of*  
837 *Marine and Freshwater Research* **34**, 155-157.
- 838 Baranski M, Rourke M, Loughnan S, Austin C, Robinson N (2006) Isolation and  
839 characterization of 125 microsatellite DNA markers in the blacklip abalone,  
840 *Haliotis rubra*. *Molecular Ecology Notes* **6**, 740-746.
- 841 Beaumont MA, Balding DJ (2004) Identifying adaptive genetic divergence among  
842 populations from genome scans. *Molecular Ecology* **13**, 969-980.
- 843 Belkhir K, Borsa P, Chikhi. L., Raufaste N, Bonhomme F (2004) GENETIX 4.05,  
844 logiciel sous Windows TM pour la génétique des populations. Laboratoire  
845 Génome, Populations, Interactions, CNRS UMR 5171, Université de  
846 Montpellier II, Montpellier

- 847 Benestan L, Gosselin T, Perrier C, *et al.* (2015) RAD genotyping reveals fine-scale  
848 genetic structuring and provides powerful population assignment in a widely  
849 distributed marine species, the American lobster (*Homarus americanus*).  
850 *Molecular Ecology* **24**, 3299-3315.
- 851 Bester-van der Merwe AE, Roodt-Wilding R, Volckaert FAM, D'Amato ME (2011)  
852 Historical isolation and hydrodynamically constrained gene flow in declining  
853 populations of the South-African abalone, *Haliotis midae*. *Conservation*  
854 *Genetics* **12**, 543-555.
- 855 Blacket MJ, Robin C, Good RT, Lee SF, Miller AD (2012) Universal primers for  
856 fluorescent labelling of PCR fragments-an efficient and cost-effective  
857 approach to genotyping by fluorescence. *Molecular Ecology Resources* **12**,  
858 456-463.
- 859 Brookfield JFY (1996) A simple new method for estimating null allele frequency  
860 from heterozygote deficiency. *Molecular Ecology* **5**, 453-455.
- 861 Brown CJ, Smith SJ, Lawton P, Anderson JT (2011) Benthic habitat mapping: A  
862 review of progress towards improved understanding of the spatial ecology of  
863 the seafloor using acoustic techniques. *Estuarine Coastal and Shelf Science*  
864 **92**, 502-520.
- 865 Brown LD (1991) Genetic variation and population structure in the blacklip abalone,  
866 *Haliotis rubra*. *Australian Journal of Marine and Freshwater Research* **42**,  
867 77-90.
- 868 Brownlow RJ, Dawson DA, Horsburgh GJ, Bell JJ, Fish JD (2008) A method for  
869 genotype validation and primer assessment in heterozygote-deficient species,  
870 as demonstrated in the prosobranch mollusc *Hydrobia ulvae*. *Bmc Genetics* **9**.
- 871 Boehm JT, Waldman J, Robinson JD, Hickerson MJ (2015) Population genomics  
872 reveals seahorses (*Hippocampus erectus*) of the western mid-Atlantic coast to  
873 be residents rather than vagrants. *PLoS One* **10**, e0116219.
- 874 Catchen J, Hohenlohe PA, Bassham S, Amores A, Cresko WA (2013) Stacks: an  
875 analysis tool set for population genomics. *Molecular Ecology* **22**, 3124-3140.
- 876 Chambers MD, VanBlaricom GR, Hauser L, Utter F, Friedman CS (2006) Genetic  
877 structure of black abalone (*Haliotis cracherodii*) populations in the California  
878 islands and central California coast: Impacts of larval dispersal and decimation  
879 from withering syndrome. *Journal of Experimental Marine Biology and*  
880 *Ecology* **331**, 173-185.

- 881 Coates JH, Hovel KA, Butler JL, Bohonak AJ (2014) Recruitment and recovery of  
882 pink abalone (*Haliotis corrugata*) in a historically overexploited kelp forest:  
883 Are local populations self-sustaining? *Journal of Experimental Marine*  
884 *Biology and Ecology* **460**, 184-192.
- 885 Colton MA, Swearer SE (2012) Locating faunal breaks in the nearshore fish  
886 assemblage of Victoria, Australia. *Marine and Freshwater Research* **63**, 218-  
887 231.
- 888 Conod N, Bartlett JP, Evans BS, Elliott NG (2002) Comparison of mitochondrial and  
889 nuclear DNA analyses of population structure in the blacklip abalone *Haliotis*  
890 *rubra* Leach. *Marine and Freshwater Research* **53**, 711-718.
- 891 Cowen RK, Paris CB, Srinivasan A (2006) Scaling of connectivity in marine  
892 populations. *Science* **311**, 522-527.
- 893 Dierickx EG, Shultz AJ, Sato F, Hiraoka T, Edwards SV (2015) Morphological and  
894 genomic comparisons of Hawaiian and Japanese Black-footed Albatrosses  
895 (*Phoebastria nigripes*) using double digest RADseq: implications for  
896 conservation. *Evolutionary Applications* **8**, 662–678.
- 897 Earl DA, Vonholdt BM (2012) STRUCTURE HARVESTER: a website and program  
898 for visualizing STRUCTURE output and implementing the Evanno method.  
899 *Conservation Genetics Resources* **4**, 359-361.
- 900 Elshire RJ, Glaubitz JC, Sun Q, *et al.* (2011) A Robust, Simple Genotyping-by-  
901 Sequencing (GBS) Approach for High Diversity Species. *Plos One* **6**.
- 902 Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of  
903 individuals using the software STRUCTURE: a simulation study. *Molecular*  
904 *Ecology* **14**, 2611-2620.
- 905 Evans B, White RWG, Elliott NG (2000) Characterization of microsatellite loci in the  
906 Australian Blacklip abalone (*Haliotis rubra*, Leach). *Molecular Ecology* **9**,  
907 1183-1184.
- 908 Foll M, Gaggiotti O (2008) A Genome-Scan Method to Identify Selected Loci  
909 Appropriate for Both Dominant and Codominant Markers: A Bayesian  
910 Perspective. *Genetics* **180**, 977-993.
- 911 Galindo J, Martinez-Fernandez M, Suarez P, *et al.* (2009) The adaptive role of  
912 Phosphoglucosmutase and other allozymes in a marine snail across the vertical  
913 rocky-shore gradient. *Biological Journal of the Linnean Society* **98**, 225-233.

- 914 Gilg MR, Hilbish TJ (2003) The geography of marine larval dispersal: Coupling  
915 genetics with fine-scale physical oceanography. *Ecology* **84**, 2989-2998.
- 916 Gilmour PW, Dwyer PD, Day RW (2013) Enhancing the agency of fishers: A  
917 conceptual model of self-management in Australian abalone fisheries. *Marine*  
918 *Policy* **37**, 165–175.
- 919 Gordon HR, Cook PA (2013) World abalone supply, markets, and pricing: 2011  
920 update. *Journal of Shellfish Research* **32**, 5-7.
- 921 Gorfine H (2002) *Assessment of the sustainability of Victorian abalone resources*,  
922 University of Technology, Sydney.
- 923 Goudet J (1995) FSTAT (version 1.2): a computer program to calculate F-statistics.  
924 *Journal of Heredity* **86**, 485-486.
- 925 Gruenthal KM, Acheson LK, Burton RS (2007) Genetic structure of natural  
926 populations of California red abalone (*Haliotis rufescens*) using multiple  
927 genetic markers. *Marine Biology* **152**, 1237-1248.
- 928 Gruenthal KM, Burton RS (2005) Genetic diversity and species identification in the  
929 endangered white abalone (*Haliotis sorenseni*). *Conservation Genetics* **6**, 929-  
930 939.
- 931 Gruenthal KM, Burton RS (2008) Genetic structure of natural populations of the  
932 California black abalone (*Haliotis cracherodii* Leach, 1814), a candidate for  
933 endangered species status. *Journal of Experimental Marine Biology and*  
934 *Ecology* **355**, 47-58.
- 935 Gutierrez-Gonzalez JL, Cruz P, del Rio-Portilla MA, Perez-Enriquez R (2007)  
936 Genetic structure of green abalone *Haliotis fulgens* population off Baja  
937 California, Mexico. *Journal of Shellfish Research* **26**, 839-846.
- 938 Hahn KO (1988) Larval development of abalone. In: *The Culture of Abalone and*  
939 *Other Marine Gastropods* (ed. Hahn KO), pp. 71-99. CRC Press, Boca Raton.
- 940 Hamer PA, Jenkins GP, Womersley BA, Mills KA (2010) *Understanding the*  
941 *ecological role of abalone in the reef ecosystem of Victoria*. Final report to  
942 Fisheries Research and Development Corporation. Project No. 2006/040. .  
943 Department of Primary Industries, Queenscliff.
- 944 Hardy OJ, Vekemans X (2002) SPAGEDi: a versatile computer program to analyse  
945 spatial genetic structure at the individual or population levels. *Molecular*  
946 *Ecology Notes* **2**, 618-620.

- 947 Hauck M, Sweijd NA (1999) A case study of abalone poaching in South Africa and  
948 its impact on fisheries management. *Ices Journal of Marine Science* **56**, 1024-  
949 1032.
- 950 Hauser L, Carvalho GR (2008) Paradigm shifts in marine fisheries genetics: ugly  
951 hypotheses slain by beautiful facts. *Fish and Fisheries* **9**, 333-362.
- 952 Hedgecock D, Li G, Hubert S, Bucklin K, Ribes V (2004) Widespread null alleles and  
953 poor cross-species amplification of microsatellite DNA loci cloned from the  
954 pacific oyster, *Crassostrea gigas*. *Journal of Shellfish Research* **23**, 379-385.
- 955 Hedrick PW (2005) A standardized genetic differentiation measure. *Evolution* **59**,  
956 1633-1638.
- 957 Hess JE, Campbell NR, Close DA, Docker MF, Narum SR (2013) Population  
958 genomics of Pacific lamprey: adaptive variation in a highly dispersive species.  
959 *Molecular Ecology* **22**, 2898-2916.
- 960 Hewitt GM (2004) Genetic consequences of climatic oscillations in the Quaternary.  
961 *Philosophical Transactions of the Royal Society of London Series B-*  
962 *Biological Sciences* **359**, 183-195.
- 963 Hoffman JI, Clarke A, Clark MS, Fretwell P, Peck LS (2012) Unexpected fine-scale  
964 population structure in a broadcast-spawning Antarctic marine mollusc. *PLoS*  
965 *One* **7**, e32415.
- 966 Huang BX, Peakall R, Hanna PJ (2000) Analysis of genetic structure of blacklip  
967 abalone (*Haliotis rubra*) populations using RAPD, minisatellite and  
968 microsatellite markers. *Marine Biology* **136**, 207-216.
- 969 Ierodiaconou D, Burq S, Reston M, Laurenson L (2007) Marine benthic habitat  
970 mapping using multibeam data, georeferenced video and image classification  
971 techniques in Victoria, Australia. *Journal of Spatial Science* **52**, 93-104.
- 972 Ierodiaconou D, Monk J, Rattray A, Laurenson L, Versace VL (2011) Comparison of  
973 automated classification techniques for predicting benthic biological  
974 communities using hydroacoustics and video observations. *Continental Shelf*  
975 *Research* **31**, S28-S38.
- 976 Jackson JBC (2010) The future of the oceans past. *Philosophical Transactions of the*  
977 *Royal Society B-Biological Sciences* **365**, 3765-3778.
- 978 Jalali MA, Ierodiaconou D, Gorfine H, Monk J, Rattray A (2015) Exploring  
979 spatiotemporal trends in commercial fishing effort of an abalone fishing zone:  
980 A GIS-based hotspot model. *Plos One*.

- 981 Johansson ML, Alberto F, Reed DC, *et al.* (2015) Seascape drivers of *Macrocystis*  
982 *pyrifera* population genetic structure in the northeast Pacific. *Molecular*  
983 *Ecology* **24**, 4866-4885.
- 984 Jombart T (2008) adegenet: a R package for the multivariate analysis of genetic  
985 markers. *Bioinformatics* **24**, 1403-1405.
- 986 Jombart T, Ahmed I (2011) adegenet 1.3-1: new tools for the analysis of genome-  
987 wide SNP data. *Bioinformatics* **27**, 3070-3071.
- 988 Karpov KA, Haaker PL, Taniguchi IK, Rogers-Bennett L (2000) Serial depletion and  
989 the collapse of the California abalone (*Haliotis* spp.) fishery. *Canadian*  
990 *Special Publication of Fisheries and Aquatic Sciences* **130**, 11-24.
- 991 Kashiwada JV, Taniguchi IK (2007) Application of recent red abalone *Haliotis*  
992 *rufescens* surveys to management decisions outlined in the California Abalone  
993 Recovery and Management Plan. *Journal of Shellfish Research* **26**, 713-717.
- 994 Lambeck K, Chappell J (2001) Sea level change through the last glacial cycle.  
995 *Science* **292**, 679-686.
- 996 Lecours V, Devillers R, Schneider DC, *et al.* (2015) Spatial scale and geographic  
997 context in benthic habitat mapping: review and future directions. *Marine*  
998 *Ecology Progress Series* **535**, 259-284.
- 999 Leiva GE, Castilla JC (2001) A review of the world marine gastropod fishery:  
1000 evolution of catches, management and the Chilean experience. *Reviews in Fish*  
1001 *Biology and Fisheries* **11**, 283-300.
- 1002 Lemer S, Rochel E, Planes S (2011) Correction Method for Null Alleles in Species  
1003 with Variable Microsatellite Flanking Regions, A Case Study of the Black-  
1004 Lipped Pearl Oyster *Pinctada margaritifera*. *Journal of Heredity* **102**, 243-  
1005 246.
- 1006 Li ZB, Appleyard SA, Elliott NG (2006) Population structure of *Haliotis rubra* from  
1007 South Australia inferred from nuclear and mtDNA analyses. *Acta*  
1008 *Oceanologica Sinica* **25**, 99-112.
- 1009 Ling SD (2008) Range expansion of a habitat-modifying species leads to loss of  
1010 taxonomic diversity: a new and impoverished reef state. *Oecologia* **156**, 883-  
1011 894.
- 1012 Loiselle BA, Sork VL, Nason J, Graham C (1995) Spatial genetic structure of a  
1013 tropical understory shrub, *Psychotria officinalis* (Rubiaceae). *American*  
1014 *Journal of Botany* **82**, 1420-1425.

- 1015 Lowe WH, Allendorf FW (2010) What can genetics tell us about population  
1016 connectivity? *Molecular Ecology* **19**, 3038-3051.
- 1017 Lynch M, Ritland K (1999) Estimation of pairwise relatedness with molecular  
1018 markers. *Genetics* **152**, 1753-1766.
- 1019 Manel S, Schwartz MK, Luikart G, Taberlet P (2003) Landscape genetics: combining  
1020 landscape ecology and population genetics. *Trends in Ecology & Evolution* **18**,  
1021 189-197.
- 1022 Martinez-Fernandez M, Bernatchez L, Rolan-Alvarez E, Quesada H (2010) Insights  
1023 into the role of differential gene expression on the ecological adaptation of the  
1024 snail *Littorina saxatilis*. *Bmc Evolutionary Biology* **10**.
- 1025 Mayfield S, McGarvey R, Gorfine HK, *et al.* (2011) Survey estimates of fishable  
1026 biomass following a mass mortality in an Australian molluscan fishery.  
1027 *Journal of Fish Diseases* **34**, 287-302.
- 1028 McShane PE, Black KP, Smith MG (1988) Recruitment processes in *Haliotis rubra*  
1029 Leach (Mollusca: Gastropoda) and regional hydrodynamics in southeastern  
1030 Australia imply localized dispersal of larvae. *Journal of Experimental Marine*  
1031 *Biology and Ecology* **124**, 175-203.
- 1032 Meirmans PG (2006) Using the AMOVA framework to estimate a standardized  
1033 genetic differentiation measure. *Evolution* **60**, 2399-2402.
- 1034 Meirmans PG, Hedrick PW (2011) Assessing population structure: F-ST and related  
1035 measures. *Molecular Ecology Resources* **11**, 5-18.
- 1036 Milano I, Babbucci M, Cariani A, *et al.* (2014) Outlier SNP markers reveal fine-scale  
1037 genetic structuring across European hake populations (*Merluccius*  
1038 *merluccius*). *Molecular Ecology* **23**, 118-135.
- 1039 Miller AD, Versace VL, Matthews TG, Montgomery S, Bowie KC (2013) Ocean  
1040 currents influence the genetic structure of an intertidal mollusc in south-  
1041 eastern Australia - implications for predicting the movement of passive  
1042 dispersers across a marine biogeographic barrier. *Ecology and Evolution* **3**,  
1043 1248-1261.
- 1044 Miller KJ, Maynard BT, Mundy CN (2009) Genetic diversity and gene flow in  
1045 collapsed and healthy abalone fisheries. *Molecular Ecology* **18**, 200-211.
- 1046 Miller KJ, Mundy CN, Mayfield S (2014) Molecular genetics to inform spatial  
1047 management in benthic invertebrate fisheries: a case study using the  
1048 Australian Greenlip Abalone. *Molecular Ecology* **23**, 4958-4975.

- 1049 Morgan LE, Wing SR, Botsford LW, Lundquist CJ, Diehl JM (2000) Spatial  
1050 variability in red sea urchin (*Strongylocentrotus franciscanus*) recruitment in  
1051 northern California. *Fisheries Oceanography* **9**, 83-98.
- 1052 Mundy C, Mayfield S, Gorfine H, Liggins G (2014) Blacklip Abalone *Haliotis rubra*.  
1053 In: *Status of key Australian fish stocks reports 2014* (eds. Flood M, Stobutzki  
1054 I, Andrews J, *et al.*). Fisheries Research and Development Corporation,  
1055 Canberra.
- 1056 Park SDE (2001) *Trypanotolerance in West African Cattle and the Population*  
1057 *Genetic Effects of Selection*, University of Dublin.
- 1058 Peakall R, Smouse PE (2006) GENALEX 6: genetic analysis in Excel. Population  
1059 genetics software for teaching and research. *Molecular Ecology Notes* **6**, 288-  
1060 295.
- 1061 Piggott MP, Banks SC, Tung P, Beheregaray LB (2008) Genetic evidence for  
1062 different scales of connectivity in a marine mollusc. *Marine Ecology Progress*  
1063 *Series* **365**, 127-136.
- 1064 Prince JD, Sellers TL, Ford WB, Talbot SR (1987) Experimental evidence for limited  
1065 dispersal of haliotid larvae (genus *Haliotis*; Mollusca: Gastropoda). *Journal of*  
1066 *Experimental Marine Biology and Ecology* **106**, 243-263.
- 1067 Prince JD, Sellers TL, Ford WB, Talbot SR (1988) Confirmation of a relationship  
1068 between the localized abundance of breeding stock and recruitment for  
1069 *Haliotis rubra* Leach (Mollusca: Gastropoda). *Journal of Experimental*  
1070 *Marine Biology and Ecology* **122**, 91-104.
- 1071 Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using  
1072 multilocus genotype data. *Genetics* **155**, 945-959.
- 1073 Queller DC, Goodnight KF (1989) Estimating relatedness using genetic markers.  
1074 *Evolution* **43**, 258-275.
- 1075 Rattray A, Ierodiaconou D, Womersley T (2015) Wave exposure as a predictor of  
1076 benthic habitat distribution on high energy temperate reefs. *Frontiers in*  
1077 *Marine Science* **2**, 1-14.
- 1078 Raymond M, Rousset F (1995) An exact test for population differentiation. *Evolution*  
1079 **49**, 1280-1283.
- 1080 Rice WR (1989) Analyzing tables of statistical tests. *Evolution* **43**, 223-225.
- 1081 Ridgway KR, Condie SA (2004) The 5500-km-long boundary flow off western and  
1082 southern Australia. *Journal of Geophysical Research-Oceans* **109**.

- 1083 Ridgway KR, Godfrey JS (1997) Seasonal cycle of the East Australian Current.  
1084 *Journal of Geophysical Research-Oceans* **102**, 22921-22936.
- 1085 Rousset F (1997) Genetic differentiation and estimation of gene flow from F-statistics  
1086 under isolation by distance. *Genetics* **145**, 1219-1228.
- 1087 Sandery PA, Kämpf J (2007) Transport timescales for identifying seasonal variation  
1088 in Bass Strait, south-eastern Australia. *Estuarine Coastal and Shelf Science*  
1089 **74**, 684-696.
- 1090 Shepherd SA, Breen PA (1992) Mortality in abalone: its estimation, variability and  
1091 causes. In *Abalone of the world* (eds Shepherd, SA, Tegner, MJ & Guzman del  
1092 Proo, SA) Blackwell, Oxford.
- 1093 Solas MR, Sepulveda RD, Brante A (2013) Genetic variation of the shell morphology  
1094 in *Acanthina monodon* (Gastropoda) in habitats with different wave exposure  
1095 conditions. *Aquatic Biology* **18**, 253-260.
- 1096 Strain EMA, Johnson CR (2012) Intensive fishing of marine consumers causes a  
1097 dramatic shift in the benthic habitat on temperate rocky reefs. *Marine Biology*  
1098 **159**, 533-547.
- 1099 Temby N, Miller K, Mundy C (2007) Evidence of genetic subdivision among  
1100 populations of blacklip abalone (*Haliotis rubra* Leach) in Tasmania. *Marine*  
1101 *and Freshwater Research* **58**, 733-742.
- 1102 Teske PR, Sandoval-Castillo J, Sasaki M, Beheregaray LB (2015a) Invasion success  
1103 of a habitat-forming marine invertebrate is limited by lower-than-expected  
1104 dispersal ability. *Marine Ecology Progress Series* **536**, 221-227.
- 1105 Teske PR, Sandoval-Castillo J, van Sebille E, Waters J, Beheregaray LB (2015b) On-  
1106 shelf larval retention limits population connectivity in a coastal broadcast  
1107 spawner. *Marine Ecology Progress Series* **532**, 1-12.
- 1108 Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) MICRO-  
1109 CHECKER: software for identifying and correcting genotyping errors in  
1110 microsatellite data. *Molecular Ecology Notes* **4**, 535-538.
- 1111 Viricel A, Rosel PE (2014) Hierarchical population structure and habitat differences  
1112 in a highly mobile marine species: the Atlantic spotted dolphin. *Molecular*  
1113 *Ecology* **23**, 5018-5035.
- 1114 Walker SJ (1999) Coupled hydrodynamic and transport models of Port Phillip Bay, a  
1115 semi-enclosed bay in south-eastern Australia. *Marine and Freshwater*  
1116 *Research* **50**, 469-481.

- 1117 Walsh PS, Metzger DA, Higuchi R (1991) Chelex 100 as a medium for simple  
1118 extraction of DNA for PCR-based typing from forensic material.  
1119 *Biotechniques* **10**, 506-513.
- 1120 Waples RS (1998) Separating the wheat from the chaff: Patterns of genetic  
1121 differentiation in high gene flow species. *Journal of Heredity* **89**, 438-450.
- 1122 Waples RS, Punt AE, Cope JM (2008) Integrating genetic data into management of  
1123 marine resources: how can we do it better? *Fish and Fisheries* **9**, 423-449.
- 1124 Weeks AR, Sgro CM, Young AG, *et al.* (2011) Assessing the benefits and risks of  
1125 translocations in changing environments: a genetic perspective. *Evolutionary*  
1126 *Applications* **4**, 709-725.
- 1127 Weir B, Cockerham C (1984) Estimating F-statistics for the analysis of population  
1128 structure. *Evolution* **38**, 1358-1370.
- 1129 Whitlock MC, McCauley DE (1999) Indirect measures of gene flow and migration: F-  
1130 ST not equal  $1/(4Nm+1)$ . *Heredity* **82**, 117-125.
- 1131 York KL, Blacket MJ, Appleton BR (2008) The Bassian Isthmus and the major ocean  
1132 currents of southeast Australia influence the phylogeography and population  
1133 structure of a southern Australian intertidal barnacle *Catomerus polymerus*  
1134 (Darwin). *Molecular Ecology* **17**, 1948-1961.
- 1135 Young M, Ierodiaconou D, Womersley T (2015) Forests of the sea: Predictive habitat  
1136 modelling to assess the abundance of canopy forming kelp forests on  
1137 temperate reefs. *Remote Sensing of Environment* **170**, 178-187.
- 1138 Zavalas R, Ierodiaconou D, Ryan D, Rattray A, Monk J (2014) Habitat Classification  
1139 of Temperate Marine Macroalgal Communities Using Bathymetric LiDAR.  
1140 *Remote Sensing* **6**, 2154-2175.

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#### 1145 **Data accessibility**

1146 All microsatellite and single nucleotide polymorphism genomic datasets generated  
1147 and analyzed in this study are available in the DRYAD archives under accession  
1148 doi:10.5061/dryad.mj714.

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#### 1150 **Author contributions**

1151 This project was conceived by ADM and DAI, and the experimental design was  
1152 assisted by ARW, AAH and HKG. AVR, CW and ADM were responsible for  
1153 generating the genetic data, and the analysis was led by ADM and GR with assistance  
1154 from AAH and ARW. ADM led the writing with assistance from all authors.

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**Table 1.** Population genetic statistics for *Haliotis rubra* sites screened with 10 microsatellite loci. Mean values over loci are presented for number of alleles (a), allelic richness (r), expected ( $H_E$ ) and observed ( $H_O$ ) heterozygosities, Hardy-Weinberg equilibrium (HWE) P values, and inbreeding coefficients ( $F_{IS}$ ) (significance after corrections for multiple comparisons indicated by bold text). Codes correspond to those provided in Figure 1 and Appendix 1.

Zone	Code	a	r	$H_E$	$H_O$	HWE	$F_{IS}$
Western	BW	8.7	7.53	0.73	0.7	>0.05	0.05
	W	7.9	6.92	0.7	0.65	>0.05	0.07
	M	8.5	7.29	0.72	0.7	>0.05	0.02
	IN	8.4	7.17	0.7	0.69	>0.05	0.02
	C	8	6.79	0.69	0.64	>0.05	0.08
	WT	8.6	7.22	0.68	0.64	<b>&lt;0.001</b>	0.07
	L	8.4	7.23	0.71	0.69	>0.05	0.02
Central	AP	7	6.28	0.68	0.63	>0.05	0.07
	PA	9	7.42	0.71	0.66	>0.05	0.06
	PP	7.8	6.79	0.69	0.69	>0.05	0.01
	SI	8.3	7.32	0.72	0.73	>0.05	-0.01
Eastern	MA	7.5	7.1	0.69	0.65	>0.05	0.07
	MAL	8.3	7.21	0.69	0.59	<b>&lt;0.001</b>	<b>0.15</b>
	GI	7.9	6.85	0.69	0.68	>0.05	0.01

**Table 2.** Per locus and global F-statistics for the Victorian state-wide (broad geographic sampling) and zone specific fine scale sampling (localized spatial sampling) for *Haliotis rubra* populations. State-wide analysis  $F_{RT}$  represents differentiation among regions (Victorian fishing zones; Western, Central and Eastern; Figure 1), while  $F_{LR}$  is an estimate of differentiation among sample locations within regions. For the fine scale analysis  $F_{RT}$  represents differentiation among reef patches, while  $F_{LR}$  is an estimate of differentiation among sites within reef patches (Figure 2). Significant genetic subdivision is denoted as \* $P < 0.05$ , \*\* $P < 0.01$

	1.H08	11.E05	2.BO1	8.F11	9.H11	17.E04	11.A07	13.C12	13.F06
<b>State wide</b>									
$F_{ST}$	0.004	0.013**	0.010**	0.021*	0.009**	0.007*	0.006**	0.000	0.022*
$F'_{ST}$	0.011	0.038*	0.055**	0.029**	0.025	0.019	0.042*	0.000	0.083*
$F_{RT}$	0.000	0.007*	0.008**	0.010*	0.006**	0.003*	0.002	0.003	0.012*
$F_{LR}$	0.004	0.006	0.002	0.012*	0.003	0.003	0.004	0.000	0.010*
<b>Fine Scale</b>									
Killarney									
$F_{ST}$	0.000	0.000	0.000	0.000	0.012	0.000	0.000	0.000	0.029*
$F'_{ST}$	0.000	0.000	0.000	0.000	0.028	0.000	0.000	0.000	0.127*
$F_{RT}$	0.000	0.000	0.002	0.000	0.003	0.009	0.012*	0.000	0.006
$F_{LR}$	0.000	0.000	0.000	0.000	0.009	0.000	0.000	0.000	0.024
Marlo									
$F_{ST}$	0.010	0.045**	0.000	0.008	0.007	0.000	0.000	0.062**	0.000
$F'_{ST}$	0.013	0.125*	0.000	0.008	0.021	0.000	0.016	0.102**	0.000
$F_{RT}$	0.005	0.051**	0.004	0.000	0.007	0.017	0.020*	0.000	0.013
$F_{LR}$	0.005	0.000	0.000	0.013	0.000	0.000	0.000	0.095**	0.000
Point Petrel									
$F_{ST}$	0.016	0.026	0.004	0.000	0.000	0.000	0.000	0.005	0.045*
$F'_{ST}$	0.007	0.067	0.000	0.000	0.000	0.000	0.000	0.000	0.142
$F_{RT}$	0.012	0.000	0.022	0.000	0.004	0.002	0.000	0.029	0.012
$F_{LR}$	0.004	0.054	0.000	0.000	0.000	0.000	0.062*	0.000	0.033

**Table 3.** Pairwise estimates of  $F_{ST}$  (below diagonal) and  $F'_{ST}$  (above diagonal) among 14 *Haliotis rubra* collection sites. Values shown in bold are significant ( $P < 0.001$ ) after 10,000 permutations and corrections for multiple comparisons. Asterix denotes sites to the east of Bass Strait.

	BW	W	M	IN	C	WT	L	AP	PADD	PPHIL	SI	MA*	MAI
BW		0.000	0.000	0.000	0.000	0.020	0.000	0.052	0.005	0.061	0.000	0.056	0.03
W	0.000		0.009	0.000	0.011	0.015	0.009	0.046	0.003	0.052	0.024	0.045	0.03
M	0.000	0.003		0.000	0.000	0.000	0.000	0.013	0.000	0.033	0.000	0.023	0.01
IN	0.000	0.000	0.000		0.000	0.000	0.000	0.041	0.007	<b>0.058</b>	0.021	0.061	0.0
C	0.000	0.003	0.000	0.000		0.000	0.019	0.040	0.002	0.061	0.000	0.053	0.03
WT	0.000	0.004	0.000	0.000	0.000		0.002	0.028	0.000	<b>0.077</b>	0.026	0.047	0.02
L	0.000	0.003	0.000	0.000	0.005	0.001		0.052	0.000	0.043	0.023	0.047	0.02
AP	0.015	0.014	0.004	0.013	0.012	0.009	<b>0.016</b>		0.029	0.052	0.060	0.043	0.01
PADD	0.001	0.001	0.000	0.002	0.001	0.000	0.000	0.009		0.043	0.000	0.017	0.04
PPHIL	0.017	0.015	0.009	0.018	0.018	<b>0.024</b>	0.013	0.016	0.013		0.063	<b>0.087</b>	<b>0.09</b>
SI	0.000	0.007	0.000	0.006	0.000	0.008	0.007	0.018	0.000	0.018		0.027	0.05
MA*	0.016	0.013	0.007	0.018	0.016	0.015	0.014	0.013	0.005	0.027	0.008		0.07
MAL*	0.009	<b>0.011</b>	0.004	0.005	0.010	0.007	0.007	0.005	0.012	<b>0.029</b>	0.016	0.023	
GI*	0.017	<b>0.019</b>	0.006	0.015	0.015	0.012	0.015	0.002	0.015	<b>0.028</b>	<b>0.019</b>	0.013	0.00

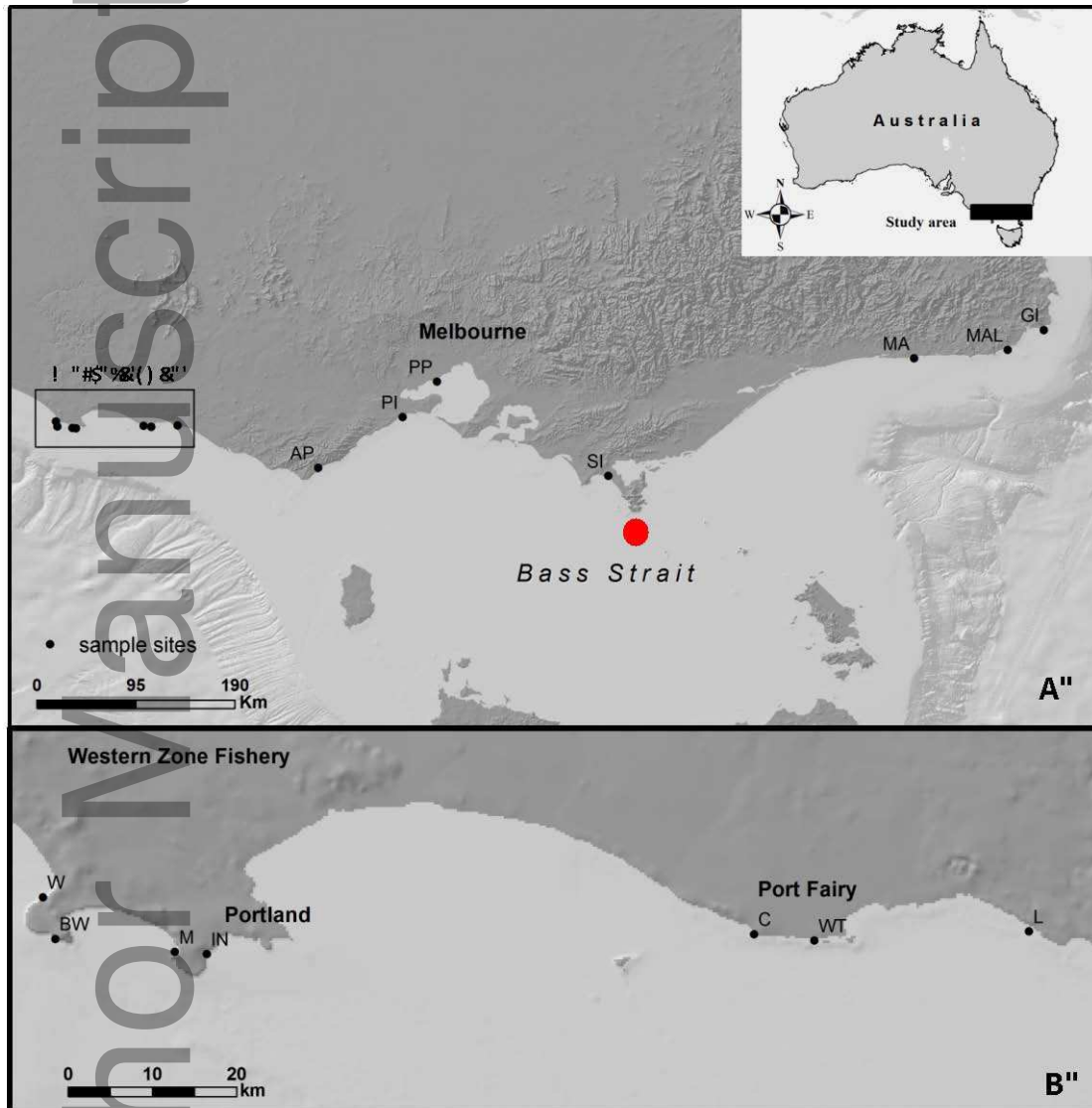
**Table 4.** Summary of tags and SNPs following filtering steps, and global estimates of observed and expected heterozygosties ( $H_O$  and  $H_E$ ), inbreeding coefficients ( $F_{IS}$ ), and population differentiation ( $F_{ST}$ ) using alternative parameter settings.  $M$  = the maximum distance allowed between stacks;  $n$  = the distance between loci in the catalogue. In all cases the minimum depth of coverage to form a stack was kept constant at  $m = 3$ .

Param	Total #	Total #	Average #	#	#SNP				
eter	l #	polymorp	tags per	polym	s				
combi	tags	hic tags	individual	orphic	MAF	H			
nation	tags	hic tags	individual	tags	>10%	$H_O$	$H_E$	$F_{IS}$	$F_{ST}$
M=7,	317	790938	126844	641	1530	0.	0.2	-0.27	0.0

n=0	865					2	0		05
	2					5			
	369					0.			
M=3,	444					2	0.1		0.0
n=0	2	976894	142821	1180	1425	4	9	-0.28	04
	231					0.			
M=3,	017					2	0.1		0.0
n=3	5	886042	144628	1121	1763	1	8	-0.17	01

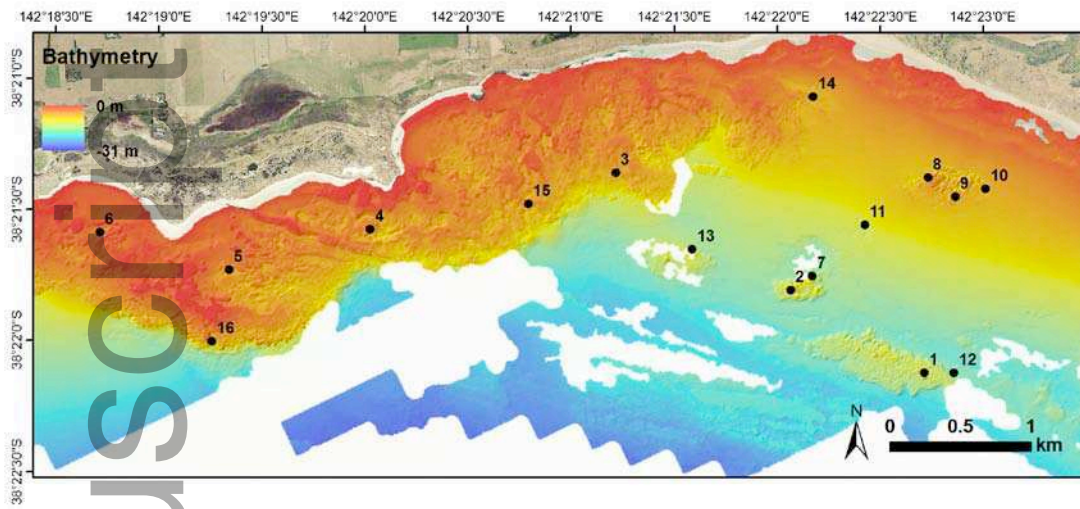
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**Figure 1.** Map of *Haliotis rubra* ‘broad geographic sampling’ collection sites from Victoria, south eastern Australia. Map ‘A’ provides a description of collection sites at a statewide scale, while map ‘B’ provides details of collection sites within the Western Zone fishery alone. All site codes correspond to those provided in table 1. The red dot indicates the biogeographic divide separating marine populations to the east and west of Wilsons Promontory.

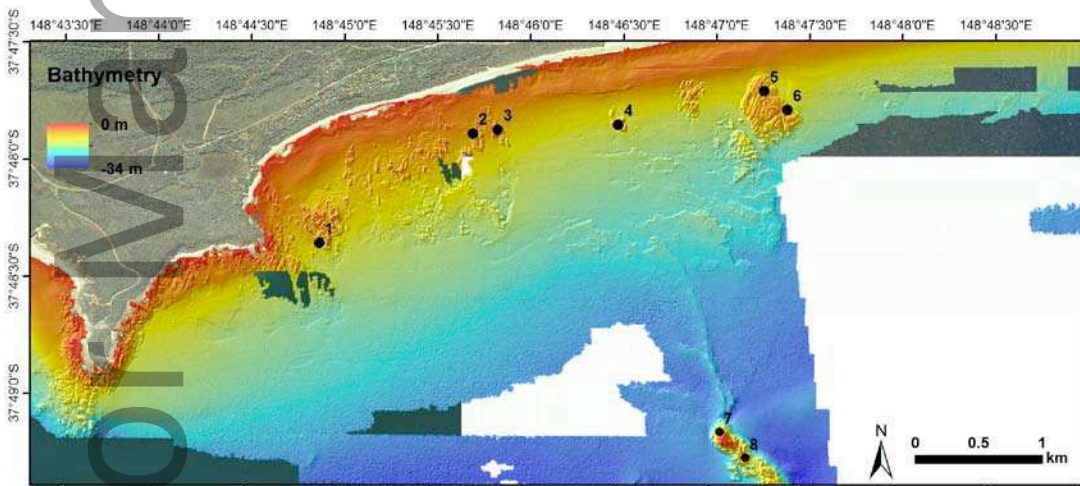


**Figure 2.** Map of *Haliotis rubra* 'localized spatial sampling' collection sites with LIDAR deduced ocean floor bathymetry. Maps A, B and C represent the Killarney, Marlo, and Petrel Point sampling locations, respectively.

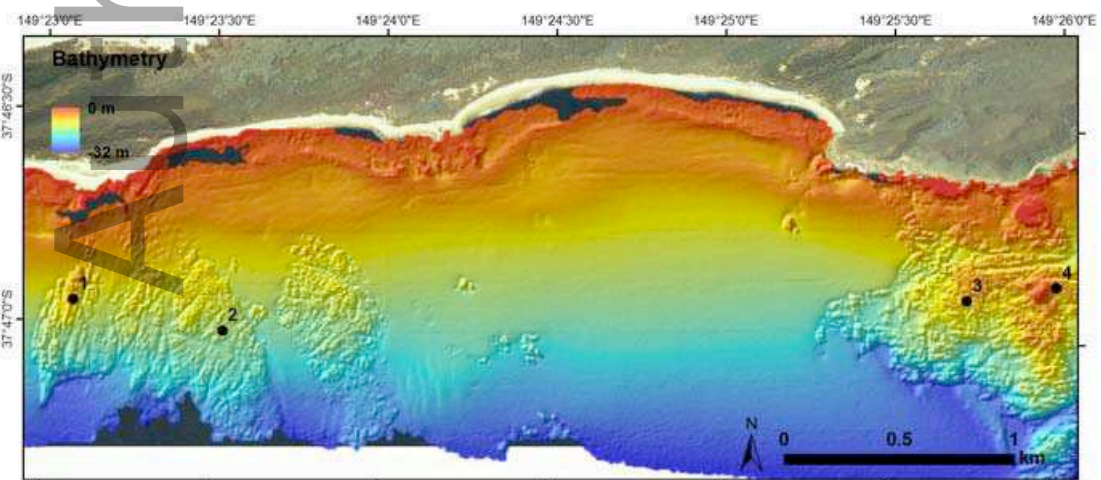
(A)



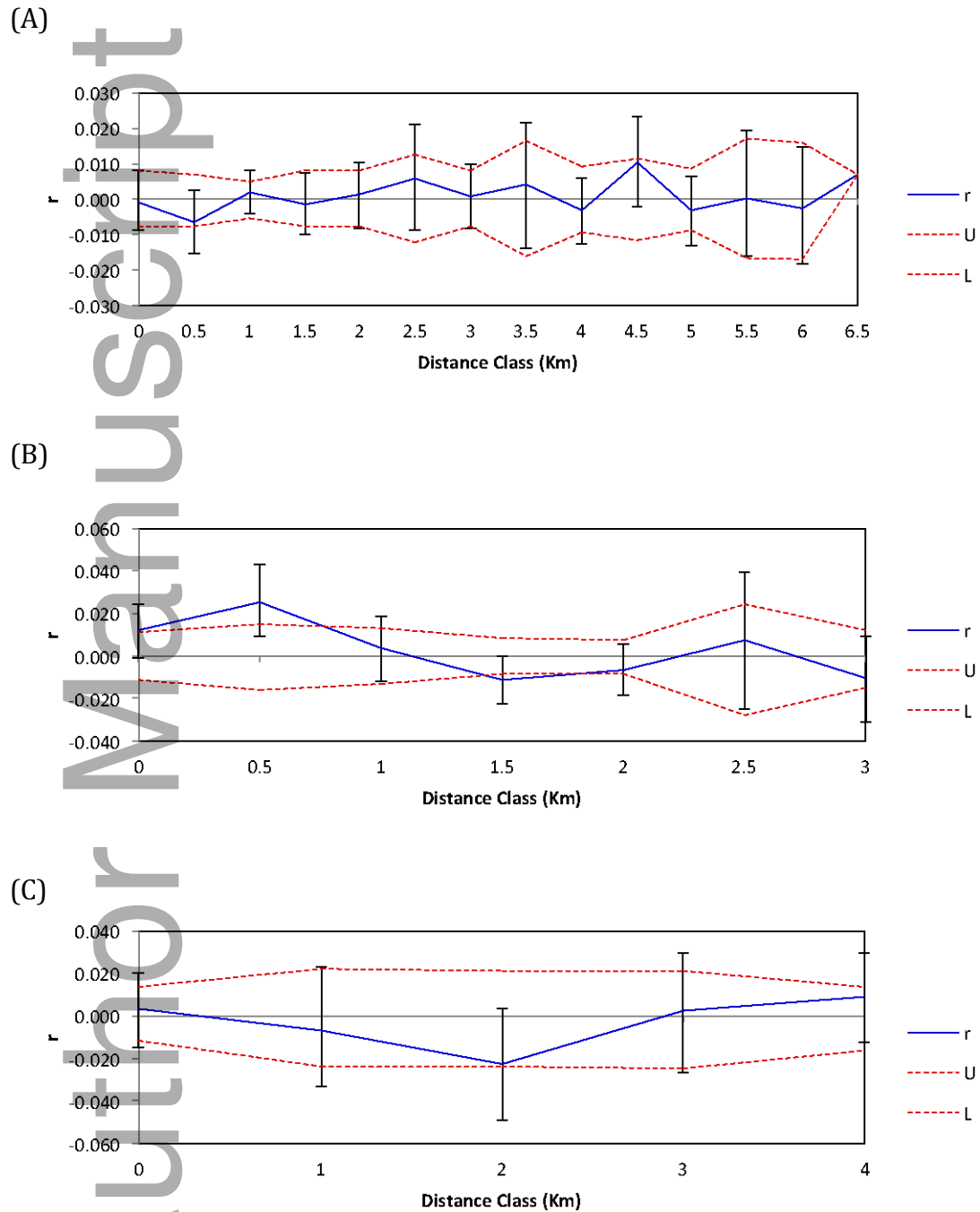
(B)



(C)



**Figure 3.** Spatial auto-correlograms of relatedness coefficient ( $r$ ) and upper (U) and lower (L) confidence intervals in relation to distance for pairs of individuals for the ‘localized spatial sampling’ experiment sites. Figures A, B and C represent the Killarney, Marlo, and Petrel Point experimental locations, respectively.



**Figure 4.** Simulated effects of varying number of *Haliotis rubra* migrants per generation ( $m$ ) on global  $F_{ST}$  among 10 sample sites from the Western and Central Zone fisheries ( $n = 10$ ) with effective population sizes ( $N_e$ ) of 500, 1000 and 10,000. The baseline empirical global  $F_{ST}$  (value 0.003) is provided as the grey dashed line.

