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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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Abstract

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

Introduction

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

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

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

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

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

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

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

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

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

Methods

Sample collections

Broad geographic sampling

Sampling across the Victorian Western, Central and Eastern Zone fisheries was conducted to determine spatial patterns of stock connectivity and genetic diversity at a large spatial scale (Figure 1; Appendix 1, see Supporting Information). *Haliotis rubra* tissue samples from the Western Zone fishery were provided by the Department of Economic Development, Jobs, Transport, and Resources (DEDJTR), Victoria, for genetic analysis. A total of 1264 whole specimens, from eight sites representing Western Zone reef codes (The Crags, The Water Tower, Murrells, Inside Cape Nelson, Levy Point, South Bridgewater, and Whites Beach) and 10 size classes (50 mm to 149 mm), were collected in 2009 by DEDJTR. Specimens from each site were collected within a 25 m radius of an anchored vessel, and subsequently preserved at -20 ° C. From these samples, we collected 50 mg biopsies from 320 individuals (40 per site), representing each of the reef codes and size classes, for genetic analysis. Dissection tools were sterilised between samples to avoid cross contamination. The biopsied material was transferred to 2 ml microcentrifuge tubes containing 100% ethanol and transported to the laboratory. Multiple size classes were sampled to avoid sampling single cohorts (potentially related individuals), which might lead to ambiguous estimates of gene flow and genetic structure. A total of 30 samples per site were used for genetic analysis. Additional samples from the Victorian Central and Eastern Zone fisheries were also included to gain insights into the broader spatial patterns of genetic connectivity (collected in 2012). Field collections and biopsies were performed following the procedures described in the previous paragraph.

LIDAR informed localized spatial sampling

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

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

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

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

DNA extraction

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

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

Microsatellite genotyping

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

Reduced representation genome library preparation

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

modified P1 and P2 adapters with 80 unique barcode combinations to allow for subsequent multiplexing of all individuals. 50 uL ligations were performed containing the MspI digested DNA, 1.125 ng of P1 and P2 adapters, 400 units of T4 ligase and 1× T4 buffer (New England Biolabs, Beverly MA, USA). Ligations were incubated at 16 ºC for 90 minutes followed by a 30 min denaturation at 80 ºC. Adapter-ligated DNA fragments were purified using a Qiagen MinElute® PCR purification kit (Redwood City, CA, USA), eluted in 20 ul of ddH_20, and subsequently used for PCR amplification. 50 uL PCRs were performed using 2 x MyTaq™ HS Mix (Bioline, Taunton, MA, USA) and containing 0.2 uM each of Illumina Dual Index Sequencing Primers 1 & 2 (Illumina Inc., San Diego, CA, USA) and 10 uL of above purified DNA. PCR conditions were: 95 ºC for 1 min, 24 cycles of 95 ºC for 30 s, 65 ºC for 30 s, 72 ºC for 30 s, and a final extension step of 72 ºC for 5 min. DNA quantitation and qualitative analysis of individual PCR products were performed on a MCE®-202 MultiNA with a DNA-1000 kit (Shimadzu, Kyoto). Samples were then pooled equimolar, and library amplicons between 250-600 bp were extracted from an agarose gel prior to sequencing on a single HiSeq™ 2000 (Illumina, San Diego) lane at the Australian National University Biomolecular Resource Facility (Australian Capital Territory, Australia).

Bioinformatics processing and genotyping

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

Microsatellite data analyses

Broad geographic sampling

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

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

Independent analyses of population structure were conducted using Bayesian analyses. Based solely on genetic data, STRUCTURE (Pritchard *et al.* 2000)

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

Localized spatial sampling

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

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

Genome-wide SNP data analyses

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

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

Population genetic simulations

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

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

Statistical power to detect genetic structure

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

Results

Microsatellite data analyses

Broad geographic sampling

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

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

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

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

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

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

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

Localized spatial sampling

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

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

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

Population genomic analysis

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

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

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

Population genetic simulations

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

Statistical power to detect genetic structure

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

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

Discussion

Spatial patterns of genetic connectivity

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

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

Fisheries management implications

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

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

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

Conclusion

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

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820
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Data accessibility

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

Author contributions

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

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	**<0.001**	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	**<0.001**	**0.15**
	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
State wide									
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*
Fine Scale									
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*	MAL
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	**0.058**	0.021	0.061	0.01
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	**0.077**	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	**0.016**		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	**0.024**	0.013	0.016	0.013		0.063	**0.087**	**0.09**
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	**0.011**	0.004	0.005	0.010	0.007	0.007	0.005	0.012	**0.029**	0.016	0.023	
GI*	0.017	**0.019**	0.006	0.015	0.015	0.012	0.015	0.002	0.015	**0.028**	**0.019**	0.013	0.00

Table 4. Summary of tags and SNPs following filtering steps, and global estimates of observed and expected heterozygosities (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.

Parameter combination	Total # tags	Total # polymorphic tags	Average # tags per individual	# polymorphic tags	#SNPs MAF >10%	H_O	H_E	F_{IS}	Global 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

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.

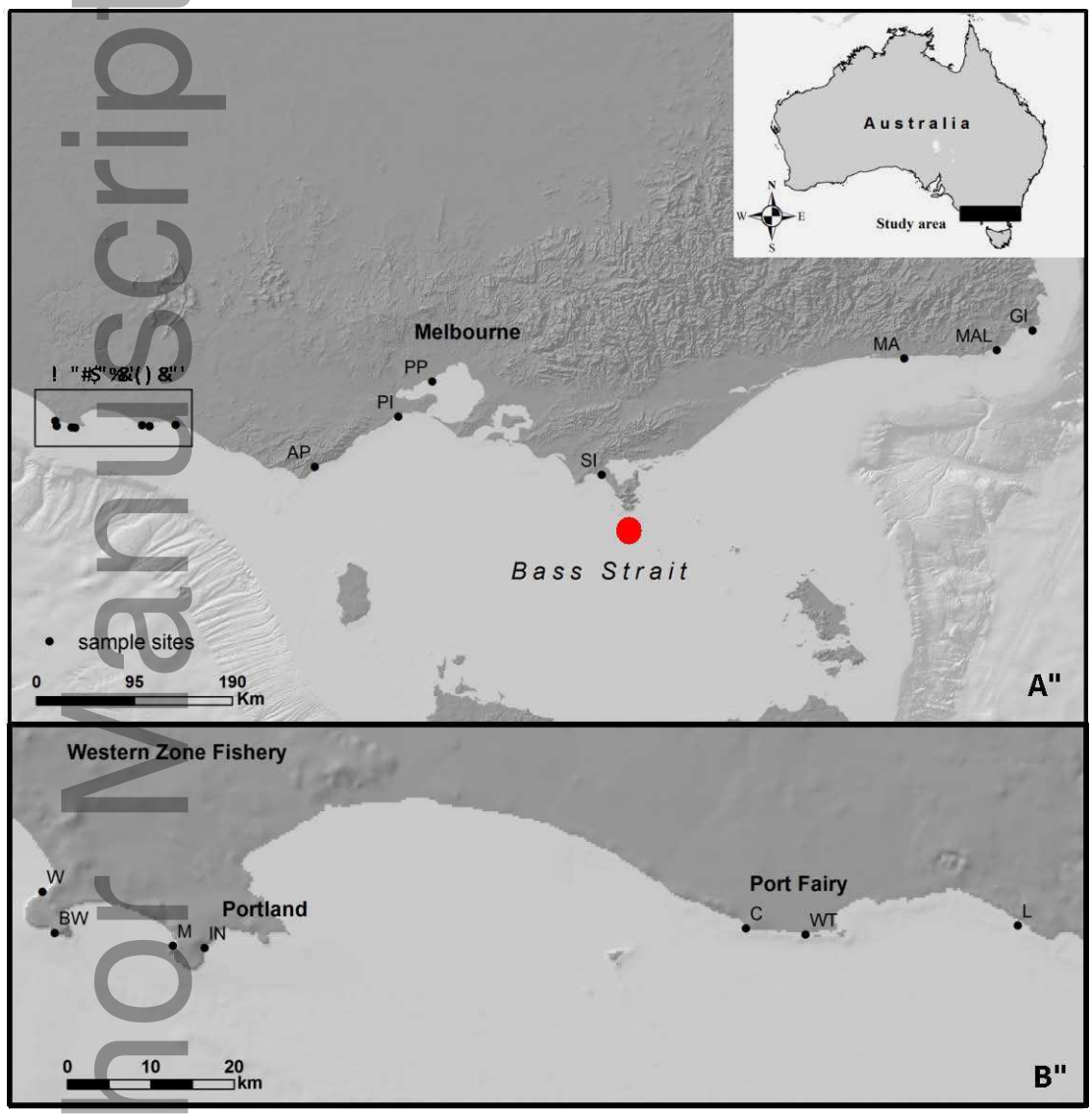
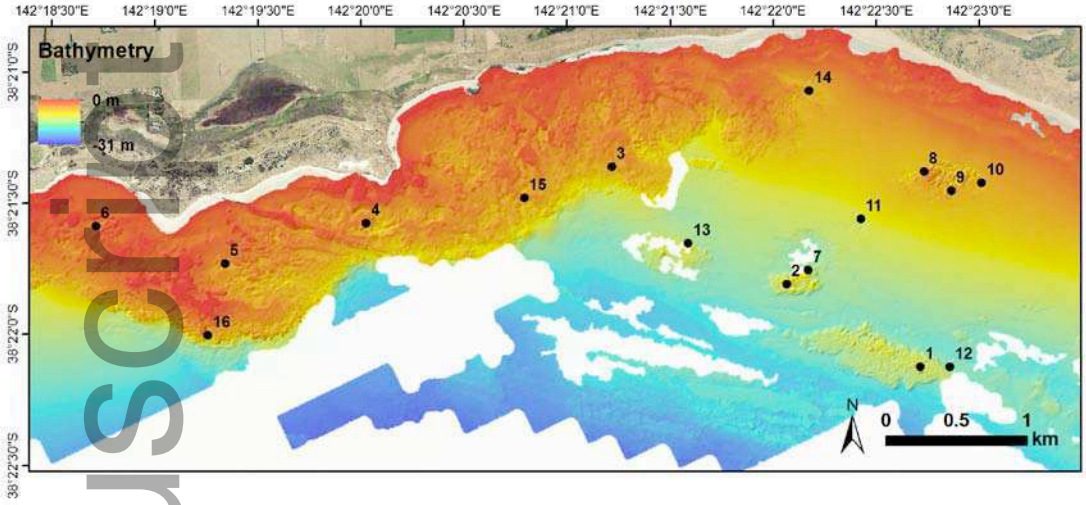
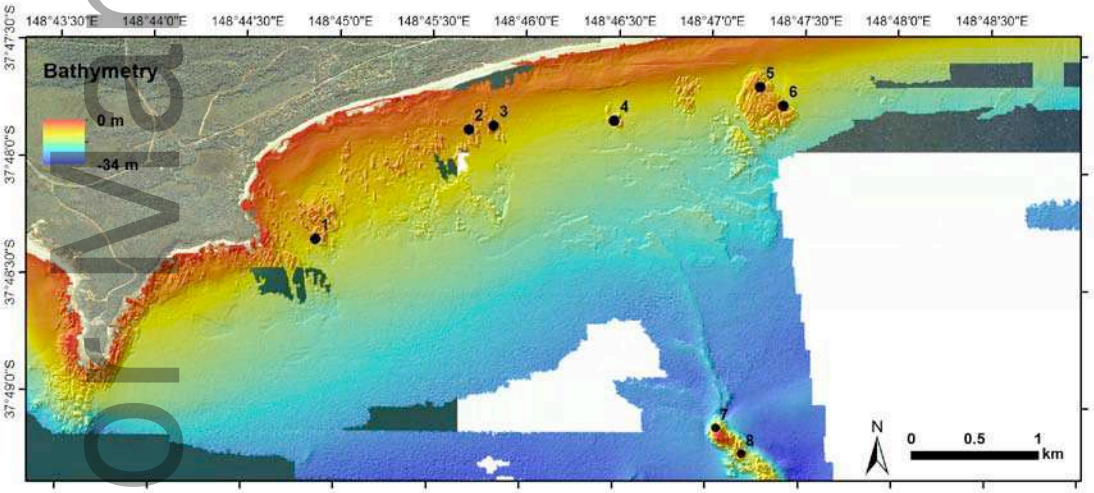


Figure 2. Map of *Haliotis rubra* 'localized spatial sampling' collection sites with LIDAR deduced ocean floor bathymetry. Maps A, B and C represent the Killarney, Marlo, and Petrel Point sampling locations, respectively.

(A)



(B)



(C)

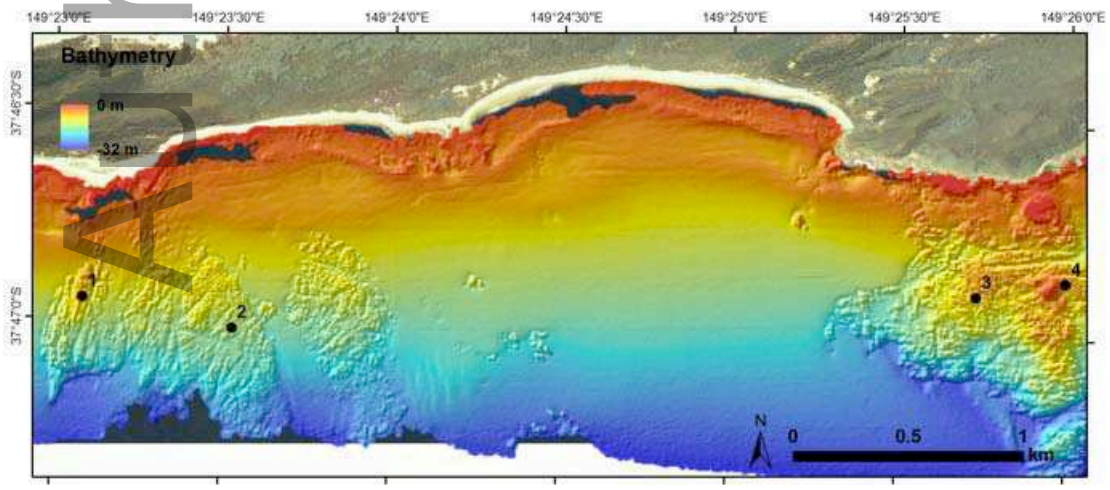
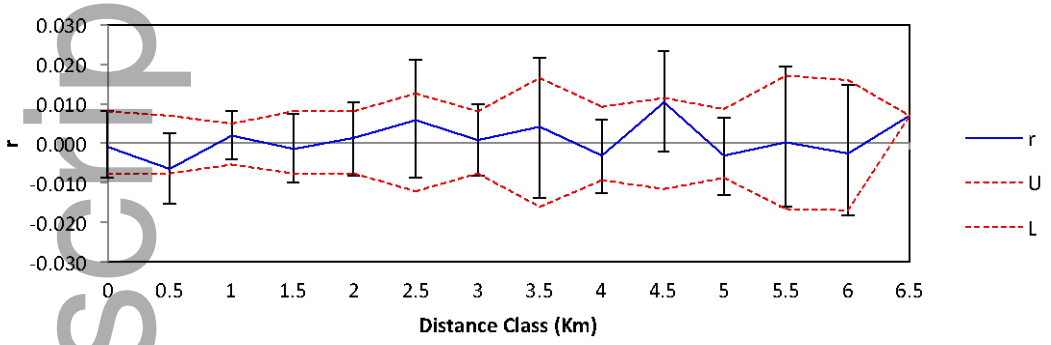
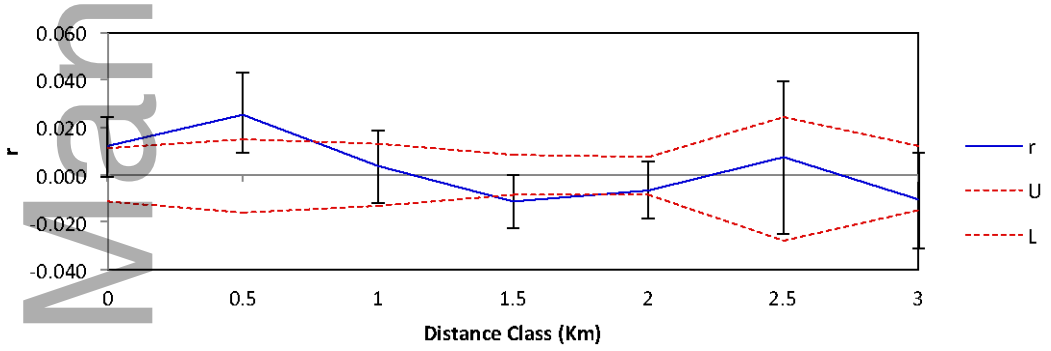


Figure 3. Spatial auto-correlograms of relatedness coefficient (*r*) and upper (U) and lower (L) confidence intervals in relation to distance for pairs of individuals for the 'localized spatial sampling' experiment sites. Figures A, B and C represent the Killarney, Marlo, and Petrel Point experimental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

(A)



(B)



(C)

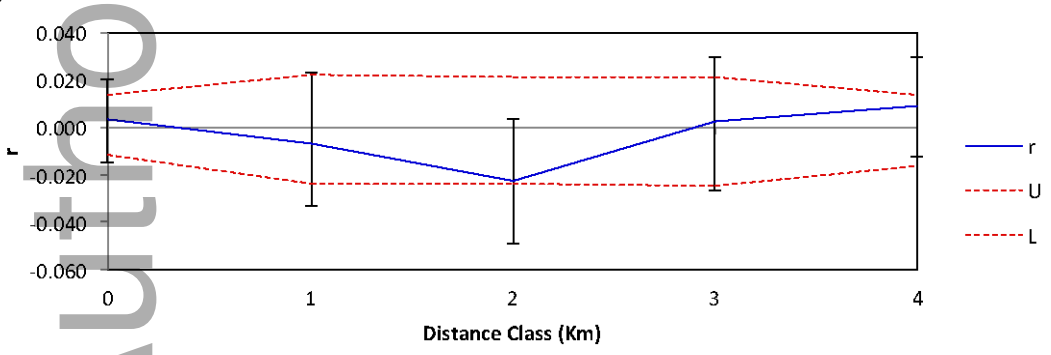
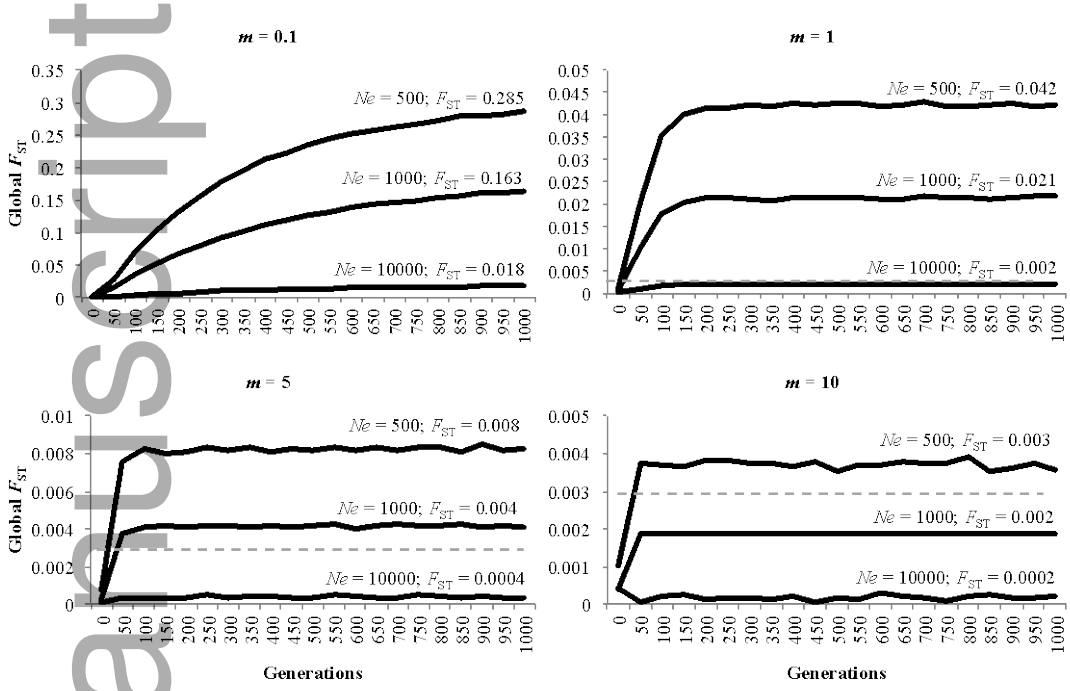


Figure 4. Simulated effects of varying number of *Haliotis rubra* migrants per generation (*m*) on global F_{ST} among 10 sample sites from the Western and Central Zone fisheries (n = 10) with effective population sizes (*Ne*) of 500, 1000 and 10,000. The baseline empirical global F_{ST} (value 0.003)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



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