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Evidence of genetic subdivision among populations of blacklip abalone (*Haliotis rubra* Leach) in Tasmania

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Abstract. The scale over which populations exchange individuals (migration) is central to ecology, and important for understanding recruitment and connectivity in commercial species. Field studies indicate that blacklip abalone (*Haliotis rubra*) have localised larval dispersal. However, genetic studies show differentiation only at large scales, suggesting dispersal over more than 100 km. Most genetic studies, however, have failed to test for subdivision at scales equivalent to field experiments. We used microsatellite DNA to investigate genetic structure at small scales (100 m to 10 km) in blacklip abalone in south-east Tasmania. We found significant subdivision ($F_{ST} = 0.021$; $P < 0.05$) among sites, and hierarchical F_{ST} analysis indicated 64% of genetic variation was at the smallest scale, supporting field studies that concluded larval dispersal is less than 100 m. We also tested if genetic differentiation varied predictably with wave exposure, but found no evidence that differences between adjacent sites in exposed locations varied from differences between adjacent sites in sheltered populations (mean $F_{ST} = 0.016$ and 0.017 respectively). Our results show the usefulness of microsatellites for abalone, but also identify sampling scales as critical in understanding gene flow and dispersal of abalone larvae in an ecologically relevant framework. Importantly, our results indicate that *H. rubra* populations are self-recruiting, which will be important for the management of this commercial species.

Additional keywords: abalone, fisheries management, gene flow, habitat, larval dispersal, microsatellite DNA.

Introduction

The link between larval dispersal, recruitment and population genetic structure is well recognised (e.g. Bohonak 1999) and has been a central tenet of ecology for decades. More recently, ecosystem changes, proposed marine protected areas and the need for ecosystem-based fisheries management all contribute to the need for a better understanding of key processes driving the population dynamics of economically and ecologically important marine species. For example, the scale and frequency of larval dispersal is seen as a critical issue in designing networks of marine protected areas (e.g. Palumbi 2003), in the debate concerning the benefits of marine protected areas beyond their boundaries (i.e. through spill-over effects: Russ and Alcala 1996; McClanahan and Mangi 2000), as well as for determining the optimum scale of fishery management (Keesing and Baker 1998).

In Tasmania, Australia, the blacklip abalone *Haliotis rubra* is the focus of the largest wild abalone fishery in the world. In addition, *H. rubra* is the dominant herbivore in shallow subtidal habitats of Tasmania, and is therefore expected to play a key role in ecosystem functioning. Sustainable management of this important fishery will hinge on a thorough understanding of the biology and ecology of *H. rubra*, especially how harvested populations are replenished either from local or distant populations, and how factors such as adult density affect fertilisation success, larval settlement and subsequent recruitment. However, aspects of the early life history of *H. rubra*, especially larval dispersal and the spatial and temporal patterns in recruitment variability, remain poorly understood (e.g. McShane 1995*a*).

Haliotis rubra is a broadcast spawner with negatively buoyant eggs. Fertilised eggs remain on or near the substrate for the first 24 h of development, following which the lecithotrophic veliger larvae are planktonic. Field-based studies have indicated that dispersal of the planktonic larvae of *H. rubra* is likely to be low (0–50 m: Prince *et al.* 1987, 1988) and hydrodynamic models also predict that recruitment in *H. rubra* will be primarily local (i.e. at scales $<100\,m^2$: McShane *et al.* 1988). However, these findings are contrary to the inferred dispersal phase of 3–15 days that has been derived from laboratory trials of settlement competency (McShane 1992). In addition, studies of larval biology in other haliotid species suggest that larval abalone will generally have the potential to disperse away from natal populations (Roberts and Lapworth 2001; Takami *et al.* 2006). Little is known of the capacity for larval behaviour to influence the dispersal phase in haliotids, although water temperature appears to be a key factor influencing developmental rate, and subsequent dispersal potential of haliotids in general (Grubert and Ritar 2004).

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Several studies have used genetic markers to examine concentrations of gene flow and infer larval dispersal in abalone species, although few generalities can be drawn. For example, strong genetic subdivision has been reported in the Californian black abalone, *Haliotis cracherodii* (Hamm and Burton 2000; Chambers *et al.* 2006), over 300 km, but there was no evidence of genetic subdivision in the green abalone, *H. fulgens*, among sites separated by 2–5 km in Baja California, Mexico (Zuniga *et al.* 2000), the red abalone *H. rufescens* between northern and southern California (Burton and Tegner 2000) or the northern abalone *H. kamtschatkana* along over 200 km of coast in British Colombia (Withler *et al.* 2003), and only weak genetic subdivision has been reported in *H. roei* and *H. laevigata* populations over distances from tens of kilometres to over 1000 km in Australia (Brown and Murray 1992; Hancock 2000; Maynard *et al.* 2004).

Even within the species *H. rubra* genetic patterns are inconsistent, with estimates of neighbourhood size ranging from 55 km (Huang *et al.* 2000) to 500 km (Brown 1991). Conod *et al.* (2002) suggested that Bass Strait represented a barrier to gene flow between Victorian and Tasmanian populations of *H. rubra* (based on mtDNA and microsatellite data), although Elliott *et al.* (2002) failed to find evidence of subdivision between Tasmanian and mainland Australian sites using just microsatellites ($F_{ST} = -0.002$, $P = 0.825$). Furthermore Elliott *et al.* (2002) found no genetic subdivision among populations around the entire coast of Tasmania ($F_{ST} = -0.029$, $P = 1.00$) and Zhongbao *et al.* (2006) reached the same conclusion about *H. rubra* from three areas in South Australia separated by up to 60 km (pairwise $F_{ST} = -0.034$–0.013, $P = 1.00$) based on microsatellites and mtDNA-RFLP. Despite little consensus among genetic studies as to neighbourhood size in *H. rubra*, interestingly all suggest gene flow and larval dispersal distances in excess of those inferred from empirical field studies and hydrodynamic modelling of dispersal (Prince *et al.* 1987, 1988; McShane *et al.* 1988).

One of the key difficulties encountered in reconciling dispersal in *H. rubra* from the genetic and ecological data in previous studies is that sampling has been at different spatial scales, and quite often in different environments. Although the ecological studies focussed on small spatial scales (i.e. <1 km), genetic studies have generally sampled larger scales, with sites separated by tens of kilometres to hundreds of kilometres, and they therefore have limited capacity to support or refute the scale of dispersal identified in field studies. In addition, the field study of Prince *et al.* (1987) was conducted in a relatively sheltered location, possibly atypical of the exposed rocky reef habitats preferred by *H. rubra*, whereas genetic studies have sampled primarily from populations on exposed coasts. Environmental factors such as wave action, wind driven currents, wave exposure, and geographic barriers will influence larval dispersal and settlement (e.g. Gaines and Roughgarden 1987; Denny and Shibata 1989; Young 1995; Bradbury and Snelgrove 2001), and the effect of environment on population genetic structure is well recognised (e.g. Ayre 1985; Johnson and Black 1991; Johannesson and Tatarenkov 1997). Certainly gene flow may be greater in exposed locations than in sheltered locations as a result of the greater potential for dispersal of gametes and larvae associated with water movement (e.g. Richards *et al.* 1995), and this may explain, at least in part, the discrepancy among ecological and genetic studies.

Here, we use microsatellite DNA markers to test for evidence of genetic subdivision among populations of *H. rubra* in southern Tasmania, specifically at the small spatial scales (metres to kilometres) used in the ecological studies of Prince *et al.* (1987, 1988) and McShane *et al.* (1988). We also compare genetic differentiation among abalone populations from exposed and sheltered habitats to determine whether *H. rubra* population structure varies predictably with exposure.

Materials and methods

Sample collection and site description

We sampled 540 abalone from three locations on the east coast of the Tasman Peninsula, south-east Tasmania (Fig. 1) from April to June 2002. Within each of the three locations (Lagoon Bay, Fortescue Bay and Port Arthur) we collected 30 abalone from each of six sites (three sites within a sheltered area (embayment) and three sites from an adjacent exposed area (open coast); a total of 18 sites overall). Sites within each location and exposure were separated by ~100–200 m and covered an area of 40–80 m^2.

A sliver of foot muscle was removed from each abalone and placed in a 1.5-mL microcentrifuge tube. Animals were returned to the site of collection after biopsy. Foot muscle tissue samples were held on ice during transportation back to the laboratory where they were stored at −20°C before DNA extraction.

Extraction of genomic DNA, PCR amplification and genotyping

Genomic DNA was extracted from foot muscle tissue using DNAzol® (Molecular Research Center Inc., Cincinatti, OH) reagent as per the manufacturer's protocol. Extracted DNA was resuspended in milli-Q water and stored at −20°C. The genomic DNA was used as a template in polymerase chain reaction (PCR) reactions to amplify three microsatellite loci; *CmrHr1.14*, *CmrHr1.24*, and *CmrHr2.14* (Evans *et al.* 2000). These loci were chosen as they appeared to be in Hardy–Weinberg equilibrium in large-scale studies and considered unlikely to have null alleles (N. Elliot, pers. comm.). A fourth microsatellite locus (RUBGACA1: Huang *et al.* 2000) was also tested but found to amplify unreliably in our samples and so was not used for the final analysis.

Polymerase chain reactions for each locus were carried out in a total volume of 20 μL and contained: 1 μL template DNA, reaction buffer (6.7 mM Tris (pH 8.8), 1.6 mM $(NH_4)_2SO_4$, 0.045% Triton X-100, 0.02 mg mL^{-1} gelatin), 200 μM each dNTP, 2.5 mM $MgCl_2$, 0.5 μM each primer, 0.05 mg mL^{-1} bovine serum albumin (BSA), and 1u TAQ Polymerase (Promega, Madison, WI). Forward primers were 5′ end-labelled with WellRED dyes (Beckman Coulter Inc., Fullerton, CA). PCR reactions were carried out in a Corbett Research Palm Cycler™ (Corbett Life Science, Sydney) with cycling conditions of 94°C for 5 min; 30 cycles of 94°C for 30 s, 60°C for 30 s, 72°C for 1 min; 72°C for 5 min for loci *CmrHr1.24* and *CmrHr2.14*. For locus *CmrHr1.14*, cycling conditions were identical except that the annealing step was 53°C for 30 s and 2 μL of template DNA were used in the reaction mix.

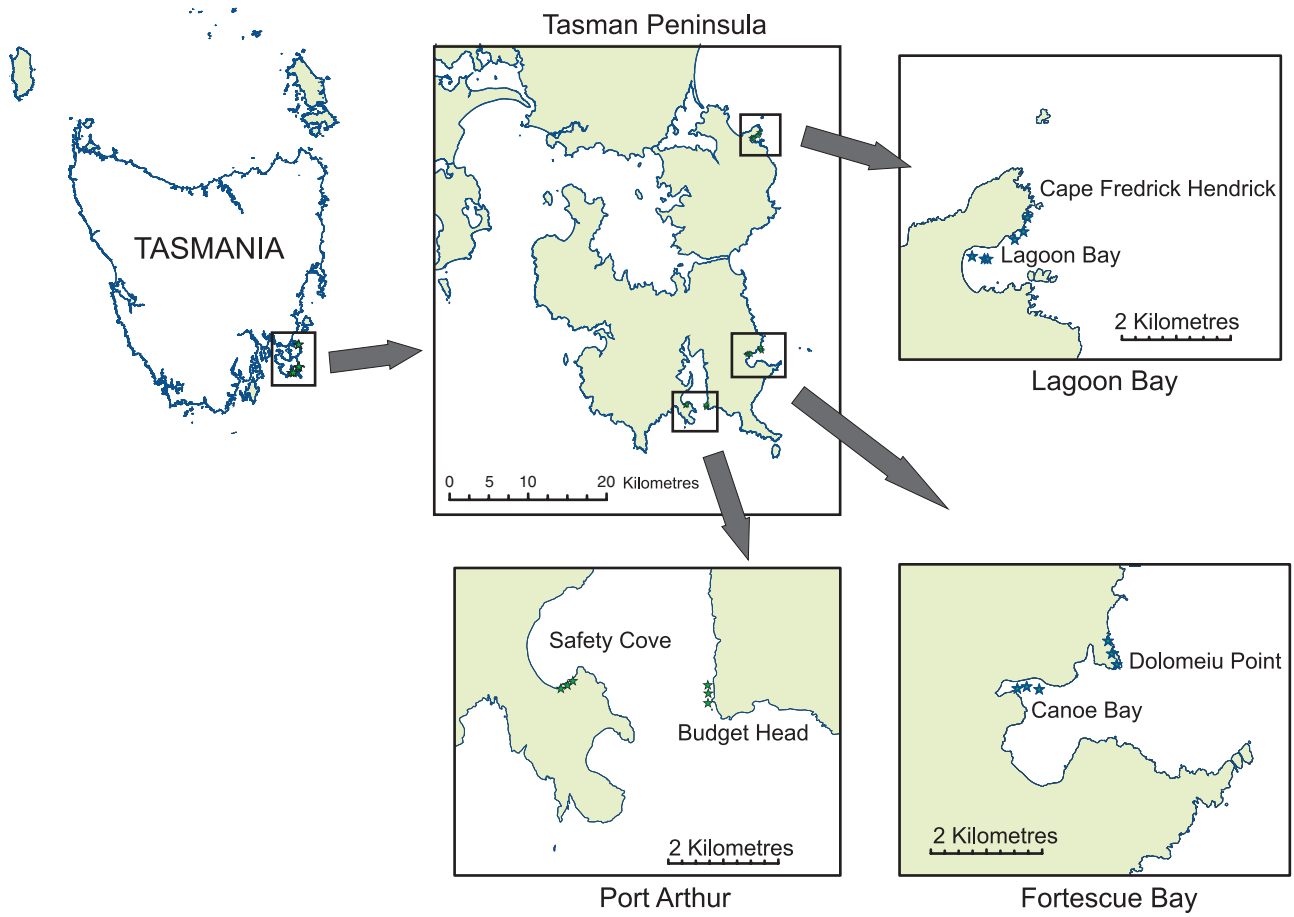

Fig. 1. Map of sample locations along the Tasman Peninsula, Tasmania, Australia.

Amplification products were visualised on 1% agarose gels, then the PCR products for the three loci were pooled for each individual and fragments sized on a Beckman Coulter CEQ 8000XL automated sequencer against the CEQ 400 size standards. Genotypes were determined using the CEQ 8000 fragment analysis software, with alleles coded according to fragment size (in base pairs).

Data analysis

We confirmed that each of the three loci were independent by testing for linkage disequilibrium using GenePop V3.4 (Raymond and Rousset 1995). Allelic diversity, allele frequencies, and observed and expected levels of heterozygosity were calculated using GenalEx V6 (Peakall and Smouse 2006) to determine levels of genetic variability within each of the sites.

We then assessed the genetic structure within *H. rubra* populations in two ways. First we determined if the frequency of genotypes at each locus matched expectations of Hardy–Weinberg equilibrium for randomly mating populations with χ^2 tests using the software GenePop v3.4 (Raymond and Rousset 1995). We then calculated values of Wright's fixation index (f) to determine if departures from equilibrium represented heterozygote deficiencies (i.e. $f > 0$) or excesses (i.e. $f < 0$). Where heterozygote deficiencies were present, we tested for the presence of null alleles using Micro-checker (van Oosterhout *et al.* 2004).

We used F-statistics, calculated as Weir and Cockerham's θ, to examine levels of genetic differentiation among sites across the Tasman Peninsula using the program Tools for Population Genetic Analyses (TFPGA: Miller 1997*b*). Mean F_{ST} was calculated by jack-knifing over loci. Departures from panmixis among sites was tested using 95% CI calculated by bootstrapping over loci. Because microsatellites invariably lead to low F_{ST} estimates as a result of high amounts of within-population genetic variation, we also calculated a standardised measure of F_{ST} (F'_{ST}) according to the methods described by Hedrick (2005) and Miermans (2006).

We used hierarchical F-statistics to partition genetic variance among sites within exposure (F_{SE}), between exposure within locations (F_{EL}), and among locations (F_{LT}). Additionally we calculated pairwise estimates of F_{ST} and used these to compare the relative levels of genetic differentiation among adjacent sites in exposed locations and among adjacent sites in sheltered locations, under the assumption that populations in exposed locations may experience higher levels of larval exchange than those in protected locations, associated with increased water movement.

Table 1. Values of Wrights fixation index (*f*) for *Haliotis rubra* populations from 18 sites along the Tasman Peninsula
Positive values of *f* represent heterozygote deficits, whereas negative values represent heterozygote excesses. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. Values in bold remain significant after Bonferroni correction for multiple simultaneous tests.

Location		Site no.	Locus		
			CmrHr1.14	*CmrHr1.24*	*CmrHr2.14*
Fortescue Bay	Canoe Bay (sheltered)	1	−0.071	0.179	0.025
		2	**0.493*****	−0.148	0.319**
		3	0.455**	−0.144	0.070
	Dolomeiu Point (exposed)	1	−0.207	0.170	0.446**
		2	0.078	0.167*	0.165
		3	0.115	−0.001	−0.067
Lagoon Bay	Lagoon Bay (sheltered)	1	0.276	0.033	0.083
		2	0.274	0.038	0.119
		3	0.222	−0.180	0.024
	Cape Frederick Hendrick (exposed)	1	0.189	0.237*	0.234**
		2	0.120	0.096	0.252
		3	0.399*	0.000	0.252**
Port Arthur	Safety Cove (sheltered)	1	0.379**	−0.045	0.205
		2	**0.538******	0.296	0.168
		3	0.197	0.170	0.140*
	Budget Head (exposed)	1	0.099	0.288**	−0.029
		2	**0.577*****	0.183	0.235
		3	**0.77*****	0.163**	0.046

Where larval dispersal is restricted, an association between genetic differentiation and distance might be expected, whereby genetic differentiation will increase with geographic distance (i.e. isolation by distance: Wright 1943). We tested for evidence of isolation-by-distance in two ways. First, we regressed N_em with the log of geographic distance (as per Slatkin 1993) where $N_em = (1/F_{ST} - 1)/4F_{ST}$, and also used Mantel's tests to test for any association between geographic and genetic distance using TFPGA (Miller 1997*b*). We also calculated N_em based on private alleles, as per Barton and Slatkin (1986) and using GenePop V3.4 (Raymond and Rousset 1995), in order to estimate levels of gene flow among populations.

Results

Genetic structure within abalone populations

Moderate to high levels of allelic diversity were found at each of the eighteen sites. Locus *CmrHr1.14* averaged 5.6 alleles per site, locus *CmrHr1.24* averaged 5.8 alleles per site and locus *CmrHr2.14* averaged 6.3 alleles per site. Levels of expected heterozygosity were also similar across all eighteen sites, ranging from 0.4137 to 0.6352 (Appendix 1).

Sixteen of the 54 single-locus tests for Hardy–Weinberg equilibrium showed a significant departure in levels of heterozygosity from those expected under Hardy–Weinberg equilibrium. All sixteen of these were a result of heterozygote deficits, and were spread uniformly across all three loci (Table 1). Analysis with Micro-checker indicated that null alleles were considered likely to be the cause of heterozygote deficits at all three loci, so null allele frequencies were estimated for each locus and the adjusted allele frequencies (calculated based on the Oosterhout correction algorithm) used subsequently for comparisons among populations.

Genetic differentiation among populations

We found evidence of genetic subdivision among abalone populations across the Tasman Peninsula. Mean F_{ST} was low, but significantly different to values expected if populations were panmictic ($F_{ST} = 0.021$, 95% CI = 0.007–0.041, Table 2). Additionally, when F_{ST} was standardised to account for high levels of within-population variation, higher levels of differentiation were apparent among the sites ($F'_{ST} = 0.468$), with similar levels of differentiation apparent at all three loci ($F'_{ST} = 0.487$, 0.596 and 0.321 at loci *CmrHr1.14*, *1.24* and *2.14* respectively).

Hierarchical analysis showed that genetic differentiation was the greatest (and significantly different to zero) at the smallest sampling scale (i.e. among replicate sites within exposure $F_{SE} = 0.024$, 95% CI = 0.009–0.047). Significant genetic differentiation was not apparent either among locations ($F_{LT} = 0.008$, 95% CI = −0.001–0.021) or between sheltered and exposed areas within a location ($F_{EL} = 0.005$, 95% CI = −0.007–0.022), and there was no evidence of isolation by distance among abalone populations (linear regression $R^2 = 0.032$; Mantel's test r = 0.179; $P = 0.97$). Estimates of the migration rate among populations based on private alleles was moderate, with the number of migrants per generation ($N_em = 12.04$).

Pairwise comparisons of F_{ST} among sites within exposure at each location showed no evidence that genetic differentiation among sites may vary predictably with exposure (e.g. greater levels of differentiation do not occur among adjacent sites in protected locations than among adjacent sites in exposed locations). The average pairwise F_{ST} among adjacent sites in sheltered areas

Table 2. Results from F-statistic analyses of *Haliotis rubra* populations from 18 sites along the Tasman Peninsula, based on data adjusted for null alleles

Results from the hierarchical analysis include: F_{LT}, differentiation among sites from different locations; F_{EL}, differentiation among sites from different exposures within a location; and F_{SE}, differentiation among sites from different exposures. $^*P < 0.05$

	Locus			Across all loci (± s.d.)
	CmrHr1.14	*CmrHr1.24*	*CmrHr2.14*	
Across all sites				
F_{ST}	0.041	0.007	0.013	0.021 (±0.01)*
Hierarchical analysis				
F_{LT}	0.021	0.006	−0.001	0.008 (±0.008)
F_{EL}	0.022	0.006	−0.007	0.005 (±0.009)
F_{SE}	0.047	0.009	0.014	0.024 (±0.012)*

was 0.016 (ranging from −0.011 to 0.040) compared with 0.017 (ranging from −0.004 to 0.083) among adjacent sites in exposed areas.

Discussion

Our genetic data from the Tasman Peninsula support the findings of ecological studies (Prince *et al.* 1987, 1988; McShane *et al.* 1988) that dispersal of *Haliotis rubra* larvae is highly localised. We found low but significant levels of genetic subdivision among all sites and importantly, hierarchical analysis showed that most genetic differentiation occurred at the smallest scale (i.e. among sites separated by 100–200 m).

Interestingly, some genetic studies have concluded that breeding populations of *H. rubra* are geographically large (e.g. >500 km; Brown 1991) and others have failed to find genetic subdivision among *H. rubra* populations around the entire coast of Tasmania (Conod *et al.* 2002; Elliott *et al.* 2002). We suggest this is related to the sampling used in the previous studies whereby abalone were often sourced through commercial catches with individuals in a single sample spread over relatively large distances (i.e. hundreds of metres), and where the smallest distance among sites was usually more than 1 km. Indeed at the largest scale in our study (i.e. among locations separated by tens of kilometres), which most likely reflects the smallest scale of other studies, we also found little evidence of genetic subdivision. This finding is contrary to expectations of an isolation-by-distance (Wright 1943) or stepping-stone model of gene flow (Kimura and Weiss 1964), whereby genetic differentiation would be expected to increase with geographic distance, although similar contrary patterns have been reported commonly in marine invertebrate taxa (e.g. Johnson and Black 1982; Hellberg 1995; Miller 1997*a*; Ayre and Hughes 2000). The most likely explanation for this pattern is that recruitment to abalone populations is primarily local, but that sufficient long-distance gene flow occurs on evolutionary time scales to prevent differentiation across the entire population. Alternative, but not mutually exclusive, explanations include stabilising selection, which may lead to genetic homogeneity over larger scales even where gene flow is low (Hellberg *et al.* 2002, although this explanation may be problematic for microsatellites, which are considered neutral markers, but see Slatkin 1995 for discussion of genetic hitch-hiking relative to microsatellites), and chaotic genetic patchiness associated with spatial and temporal variation in recruitment leading to large-scale uniformity despite fine-scale genetic patchiness (Johnson and Black 1982). Importantly it is the ecological processes that will be more relevant in terms of effective present-day management of abalone populations, which emphasises the importance of sampling across multiple spatial scales in order to fully understand the spatial structure of populations.

Our F_{ST} values are relatively low (Table 2), although the interpretation of microsatellite results must consider the effect of high polymorphism (as a result of mutations), which drastically deflates F_{ST} expectations (Balloux and Lugon-Moulin 2002). Hence, a seemingly low F_{ST} value (<0.05) may in fact indicate important genetic differentiation, as is evident in our significant departures from panmixis. Certainly when we adjusted F_{ST} to account for high levels of within-population variation, genetic differentiation among sites was high ($F'_{ST} = 0.47$).

In the present study we were only able to analyse data from three of the four loci that we tested; but these loci display good levels of variability with 11, 13 and 17 alleles respectively. Certainly the total number of alleles scored, rather than just the number of loci examined affects the precision of genetic distance estimates (e.g. Kalinowski 2002), so our data might be expected to give reasonable estimates of genetic differentiation in *H. rubra* despite few loci being examined. Importantly, our F_{ST} values for two of the three loci in the Tasman Peninsula sites were in fact higher than those reported across all of Tasmania for the same loci by Elliott *et al.* (2002) (Elliott *et al.* 2002 compared with the present study: *CmrHr1.14* 0.005 *v.* 0.041; *CmrHr2.14* 0.006 *v.* 0.013), which emphasises the importance of a fully replicated, hierarchical sampling design so as to accurately detect the relevant scale of population subdivision. Interestingly though, despite having chosen the three loci of Evans *et al.* (2000) that were considered most likely to be in Hardy–Weinberg equilibrium, all three loci in the present study still showed evidence of heterozygote deficits and were considered likely to have null alleles. This trend is becoming increasingly apparent in studies of marine invertebrates using microsatellite markers, and is likely to be an important source of genotyping errors.

The variation in results between our study and those of other genetic studies of *H. rubra* is also likely to be related to sample

size and ability to detect rare alleles that contribute to most of the variation among populations. For example, Huang *et al.* (2000) sampled only 10 individual abalone at each of their 10 locations in Victoria and New South Wales, which may well have reduced the power to determine the true scale of population subdivision. Equally, although Elliott *et al.* (2002) sampled considerably more abalone across all of Tasmania than we did (total $n = 3083$ compared with 540 in the present study), at the smaller or regional scale where data can be compared from nearby localities, their sample size was actually smaller than ours (e.g. $n = 300$ across the entire Bruny Bioregion for Elliott *et al.* 2002 compared with $n = 540$ for the Tasman Peninsula, which is only one part of the Bruny Bioregion). As would be expected, larger sample sizes will contain a higher proportion of rare alleles, which will increase the power of a study to detect genetic differentiation. Indeed estimates of migration rates based on rare alleles suggested only 12 migrants would be exchanged across our sites per generation. Although this is enough to counteract population divergence through genetic drift (i.e. $N_em > 1$), it is at the lower end of the range recorded across many marine invertebrate species with pelagic larvae (e.g. scleractinian corals $N_em = 3$–32: Ayre and Hughes 2004; zoanthids $N_em = 5.6$–35.5: Burnett *et al.* 1995; clams $N_em \geq 20$: Benzie 1993; *Haliotis kamtschatkana* $N_em = 18.7$: Withler *et al.* 2003; the gastropod *Chorus giganteus* $N_em = 4.75$: Gajardo *et al.* 2002).

Genetic differentiation among populations of benthic marine invertebrates is considered to be negatively correlated with larval dispersal capability (e.g. Bohonak 1999). In *H. rubra*, spawning has only been observed during calm weather (Breen and Adkins 1980) and it has been suggested that spawning will coincide with periods of low water movement (Prince *et al.* 1987). In addition, eggs are negatively buoyant and the early stages of development occur on or near the benthos. Taken together, these factors suggest gene flow and larval dispersal should be low. McShane *et al.* (1988) carried out extensive plankton tows to obtain *H. rubra* larvae on Victorian reefs, and found only a single larva throughout his study. As sampling was conducted at a time when *H. rubra* was reproductively active and when recent post-larvae where found on reef substrate at the same sites, this suggests that the larvae are being entrained within the reef habitat, which is consistent with evidence of genetic subdivision at local scales found in our study. Interestingly, we found no evidence that genetic differentiation varied predictably with water movement (based on site exposure) even though the swimming behaviour of abalone larvae is thought to confer limited directional movement in the sea (McShane 1992) and we might expect dispersal to be greater where water movement is high. It may be that factors other than water movement have greater influence over dispersal, and that abalone larvae do not simply disperse as passive particles.

For benthic mobile species such as abalone, movement of adults might be expected to negate the genetic effect of limited larval dispersal at least on small scales. However, movement of haliotids is generally limited to movement within or among patches (meters or tens of metres), with little interchange of adults between local populations (Morgan and Shepherd 2006). Thus, the genetic differentiation we have found among populations of *H. rubra* along the Tasman Peninsula most likely reflects limited movement of adults coupled with localised recruitment of larvae.

Implications for management of abalone fisheries

Our study of population structure and gene flow in *Haliotis rubra* from the Tasman Peninsula has revealed significant population subdivision at small scales (<200 m). This result supports the conclusions based on ecological field studies that larval dispersal and recruitment of the blacklip abalone *H. rubra* is primarily local (Prince *et al.* 1987, 1988; McShane *et al.* 1988), and also suggests microsatellite markers will be useful for understanding population and stock structure of abalone. Recent development of additional microsatellite loci for *H. rubra* (Baranski *et al.* 2006) will provide further opportunities for genetic research on this commercially important species that will be valuable for fisheries management.

Our results will also have important ramifications for management of abalone fisheries in Australia. *H. rubra* is patchily distributed at a range of scales from metres to hundreds of metres (Shepherd and Brown 1993; McShane 1995*b*; Morgan and Shepherd 2006) and consequently wholesale removal of reproductively mature individuals through fishing, combined with their naturally low movement patterns (juvenile and mature individuals show limited emigration from sites: Prince 1989; Shepherd and Godoy 1989; Dixon *et al.* 1998) and self-recruitment, increase the risk of localised population collapse. In addition, recovery following commercial removal will be slow, and largely dependent on a local source of larvae for replenishment of those populations. There is a long history of collapse in abalone fisheries around the world (Richards and Davis 1993; Shepherd *et al.* 1998, 2001; Karpov *et al.* 2000), with efforts to rebuild depleted stocks proving to be a major challenge (Campbell 2000; Campbell *et al.* 2000; Seki and Taniguchi 2000; Shepherd *et al.* 2000; Tegner 2000). Limited dispersal of abalone larval, juvenile and adult stages will limit the usefulness of marine protected areas or harvest refugia for conservation of these species. Integration of life histories and ecological knowledge into fishery management plans will therefore be essential to ensure sustainable management of key invertebrate herbivores such as haliotids.

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Appendix 1. Allele frequencies at three microsatellite loci for *Haliotis rubra* populations from 18 sites along the Tasman Peninsula, Tasmania, Australia

Allele	Fortescue Bay						Lagoon Bay						Port Arthur					
	Canoe Bay (sheltered)			Dolomeiu Point (exposed)			Lagoon Bay (sheltered)			Cape Frederick Hendrick (exposed)			Safety Cove (sheltered)			Budget Head (exposed)		
	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3
Locus *CmrHr1.14*																		
251	0.067	0.133	0.083	0.067	0.133	0.017	0.050	0.067	0.117	0.133	0.150	0.133	0.133	–	0.050	0.150	0.100	0.033
253	–	–	–	–	–	–	–	–	–	–	–	–	–	0.050	0.050	–	–	0.033
257	0.067	–	–	0.067	0.067	0.050	–	0.017	0.050	–	–	0.033	–	0.083	0.017	0.050	0.017	0.050
259	0.767	0.767	0.750	0.717	0.683	0.750	0.817	0.833	0.700	0.717	0.783	0.717	0.617	0.750	0.700	0.650	0.500	0.633
261	0.067	0.100	0.117	0.117	0.117	0.167	0.083	0.050	0.050	0.133	0.067	0.067	0.150	0.067	0.117	0.067	0.200	0.167
263	0.017	–	–	–	–	–	–	–	0.050	–	–	–	0.033	–	–	0.017	–	–
267	0.017	–	–	0.017	–	–	0.017	–	–	–	–	–	0.017	0.033	–	–	0.017	0.017
269	–	–	–	0.017	–	–	–	–	–	–	–	–	–	–	–	–	–	–
271	–	–	–	–	–	–	–	–	–	–	–	–	–	0.017	–	–	–	–
273	–	–	0.017	–	–	–	–	–	–	–	–	–	–	–	–	–	0.067	–
275	–	–	0.033	–	–	–	0.033	–	0.033	–	–	0.033	0.033	–	–	–	–	–
277	–	–	–	–	–	–	–	0.033	–	0.017	–	–	–	–	0.050	0.050	0.067	0.067
279	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.017	0.017	0.017	–
281	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.017	–
285	–	–	–	–	–	–	–	–	–	–	–	0.017	–	–	–	–	–	–
287	–	–	–	–	–	0.017	–	–	–	–	–	–	–	–	–	–	–	–
291	–	–	–	–	–	–	–	–	–	–	–	–	0.017	–	–	–	–	–
Locus *CmrHr1.24*																		
210	0.017	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
214	0.017	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
216	–	–	0.017	–	–	–	0.033	–	–	–	–	0.033	–	–	–	0.033	0.017	–
218	–	–	0.017	–	–	0.033	–	–	–	0.033	–	–	0.017	–	0.017	–	0.017	0.033
220	0.017	0.050	0.033	0.017	0.050	0.017	0.033	–	0.017	0.083	–	0.017	0.033	0.017	0.033	0.067	0.067	0.050

(*Continued*)

Appendix 1. (Continued)

Allele	Fortescue Bay						Lagoon Bay						Port Arthur					
	Canoe Bay (sheltered)			Dolomeiu Point (exposed)			Lagoon Bay (sheltered)			Cape Frederick Hendrick (exposed)			Safety Cove (sheltered)			Budget Head (exposed)		
	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3
222	0.700	0.783	0.783	0.767	0.683	0.767	0.833	0.833	0.767	0.783	0.867	0.750	0.783	0.817	0.717	0.600	0.767	0.683
224	0.133	0.083	0.100	0.100	0.083	0.117	0.050	0.117	0.117	0.067	0.100	0.067	0.050	0.050	0.067	0.167	0.050	0.133
226	0.117	0.067	0.033	0.083	0.100	0.067	0.033	0.050	0.050	0.033	0.017	0.117	0.067	0.067	0.050	–	0.067	0.033
228	–	0.017	0.017	0.033	0.033	–	0.017	–	0.050	–	0.017	–	0.033	0.017	0.017	0.033	0.017	–
230	–	–	–	–	0.050	–	–	–	–	–	–	0.017	0.017	–	0.017	–	–	–
234	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.017
236	–	–	–	–	–	–	–	–	–	–	–	–	–	0.033	0.067	0.100	–	0.050
238	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.017	–	–	–
Locus *CmrHr2.14*																		
200	–	–	–	–	–	–	–	–	–	–	–	–	–	0.017	–	–	–	–
208	0.033	–	–	–	0.100	–	–	–	–	–	–	0.050	–	–	–	–	–	–
212	0.017	0.050	–	0.033	0.017	–	–	–	0.033	0.017	0.033	0.100	0.017	0.033	0.033	0.017	0.033	0.033
216	0.150	0.033	0.050	0.017	0.017	0.117	0.067	0.067	0.050	0.083	0.100	0.033	0.100	0.017	0.067	0.050	0.050	0.067
220	–	–	–	–	–	–	–	0.017	–	–	–	0.017	–	–	–	–	–	0.017
224	0.450	0.467	0.650	0.650	0.567	0.383	0.467	0.533	0.667	0.583	0.450	0.417	0.450	0.517	0.450	0.317	0.633	0.500
228	0.117	0.300	0.183	0.183	0.117	0.267	0.200	0.117	0.100	0.167	0.217	0.200	0.233	0.183	0.150	0.150	0.183	0.183
232	–	0.017	–	–	0.017	0.050	0.033	0.050	0.017	–	0.017	–	0.017	0.033	0.017	0.067	0.017	0.017
236	0.233	0.133	0.117	0.117	0.167	0.167	0.233	0.200	0.133	0.133	0.183	0.183	0.183	0.167	0.200	0.317	0.083	0.150
240	–	–	–	–	–	0.017	–	0.017	–	0.017	–	–	–	0.033	0.083	0.067	–	0.033
244	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.017	–	–
n/locus	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30
Ho	0.511	0.367	0.389	0.400	0.478	0.522	0.344	0.344	0.367	0.422	0.378	0.539	0.444	0.333	0.478	0.578	0.356	0.411
He	0.527	0.476	0.439	0.462	0.545	0.512	0.435	0.414	0.468	0.477	0.434	0.533	0.551	0.472	0.561	0.635	0.548	0.585