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Aletta E. Bester-van der Merwe, Rouvay Roodt-Wilding, Filip Volckaert, Maria Eugenia D'Amato

Institutions: Stellenbosch University, Katholieke Universiteit Leuven, University of the Western Cape

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RESEARCH ARTICLE

Historical isolation and hydrodynamically constrained gene flow in declining populations of the South-African abalone, *Haliotis midae*

Aletta E. Bester-van der Merwe · Rouvay Roodt-Wilding · Filip A. M. Volckaert · Maria E. D'Amato

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Abstract Over the past two decades, the South African abalone (*Haliotis midae*), has been under serious threat mainly due to overexploitation. To assure successful management and conservation of wild stocks, the consideration of species-specific evolutionary and population dynamic aspects is critical. In this study, eight microsatellites and 12 single nucleotide polymorphic loci (SNPs) were applied to determine genetic structure in nine populations sampled throughout the species' natural distribution range. It spans along three biogeographical regions of the South African coastline: temperate in the West coast, warm temperate in the South coast and subtropical in the East coast. Data analysis applying frequentist and Bayesian-based clustering methods indicated weak genetic differentiation between populations of the West, South and East coast. Spatial Bayesian inference further revealed clinal variation along a longitudinal gradient and a transitional zone in the South coast. Coalescent analysis of long-term migration showed restricted interchange among the sampling locations of the South coast while estimates of effective population size were comparable between coastal regions. Furthermore demographic analysis of microsatellite data suggested population expansion, probably reflecting range expansion that occurred following glacial retreat during the Pleistocene. Overall, population structure analysis suggested contemporary (hydrographical conditions) as well as historical (Pleistocene contraction of habitat) restrictions to gene flow. This study provides the foundation for the establishment of an integrated management policy for preserving the natural diversity and adaptive potential of *H. midae*.

Keywords Conservation · Dispersal · *Haliotis midae* · Hydrodynamics · Microsatellites · Population differentiation · SNPs

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A. E. Bester-van der Merwe · R. Roodt-Wilding
Molecular Aquatic Research Group, Department of Genetics, Stellenbosch University, Stellenbosch 7600, South Africa

F. A. M. Volckaert
Laboratory of Animal Diversity and Systematics, Katholieke Universiteit Leuven, 3000 Leuven, Belgium

M. E. D'Amato
Forensic DNA Laboratory, Department of Biotechnology, University of the Western Cape, Belville 7535, South Africa

A. E. Bester-van der Merwe (✉)
Department of Genetics, Stellenbosch University, Private Bag X1, Matieland 7602, South Africa
e-mail: aeb@sun.ac.za

Introduction

In recent years, overexploitation of natural marine resources has become a leading environmental as well as social-economic problem world-wide (Mora et al. 2009). Since an array of management actions are urgently needed to either avoid collapse or stimulate restoration of vulnerable species, stock assessments of these species are now more important than ever before. Not only is it fundamental to the preservation of genetic diversity (Kenchington et al. 2003) but it also has important implications for broodstock collection and breeding programs within associated stock enhancement efforts (Ward 2006). The exploitation and decline of wild abalone stocks in particular is a world-wide phenomenon (Karpov et al. 2000) and several species have been recognized as vulnerable or endangered. *Haliotis*

sorenseni or white abalone became the first marine invertebrate species to be listed as an endangered species by the United States Fish and Wildlife Service (USFWS) (Hobday et al. 2001).

Haliotis midae, the South African abalone also known as perlemoen, is one of 56 recognised abalone species belonging to the gastropod family Haliotidae (Geiger 2000). It is a southern temperate species with a wide coastal distribution along the African shores of approximately 1,500 km. Because of its size, meat quality and market value, it is considered as one of the most sought-after and valuable marine molluscs of the African continent. Consequently, it suffers from overexploitation and together with ecological factors such as changes in habitat quality and predation pressure, has declined significantly over the last few decades (Hauck and Sweijd 1999; Day and Branch 2002). The species has also been listed in CITES Appendix III in 2007 but has since been removed. In complement of closure of the fishery in February 2008 and various other compliance efforts, the wild stocks are in need of additional management strategies. Similarly, the aquaculture industry of South Africa depends on commercially cultured abalone to supply the overseas market, which now also demands effective monitoring and control. It is therefore vital to understand the population genetic processes and historical factors responsible for the contemporary patterns of genetic diversity in wild populations.

Haliotis midae is a dioecious broadcast spawner with a seasonal reproductive cycle that only reaches sexual maturity after 8–10 years (Branch et al. 2002). They are benthic invertebrates with a non-feeding pelagic larval phase of approximately 7 days; the exact duration depends primarily on water temperature and not so much on the availability of nutrients, as is the case with feeding larvae. Although the larval phase is relatively short, larvae may travel hundreds of kilometres before settlement. As expected, little to no genetic structuring has been reported for many abalone species: *H. asinina* (Tang et al. 2004), *H. corrugata* (Díaz-Viloria et al. 2009), *H. cracherodii* (Gruenthal and Burton 2008), *H. kamtschatkana* (Withler et al. 2003), *H. midae* (Evans et al. 2004), *H. rubra* (Temby et al. 2007) and *H. rufescens* (Gruenthal et al. 2007). For *H. midae* specifically, the main oceanographic feature that could influence larval dispersal and subsequent population structuring is the occurrence of major currents along the South African coastline (Turpie et al. 2000): the cold northward Benguela current on the West coast and the warm southward Agulhas current on the East coast (Fig. 1). The region between Cape Point and Agulhas experiences large temperature fluctuations associated with upwelling of the Benguela current, whereas a persistent thermal front has been found in the Algoa Bay region just east of Port Elizabeth as a result of the inshore edge of the Agulhas current (Beckley and van Ballegooyen 1992; Lutjeharms and Ansorge 2001).

Oceanographic conditions and associated ecological factors determine three biogeographical regions along the South African coast: cool-temperate, warm-temperate and the sub-tropical (Emanuel et al. 1992) with growing evidence for genetic discontinuity in diverse intertidal and shallow water species between these regions. Main barriers have been identified in the regions of Cape Point, Cape

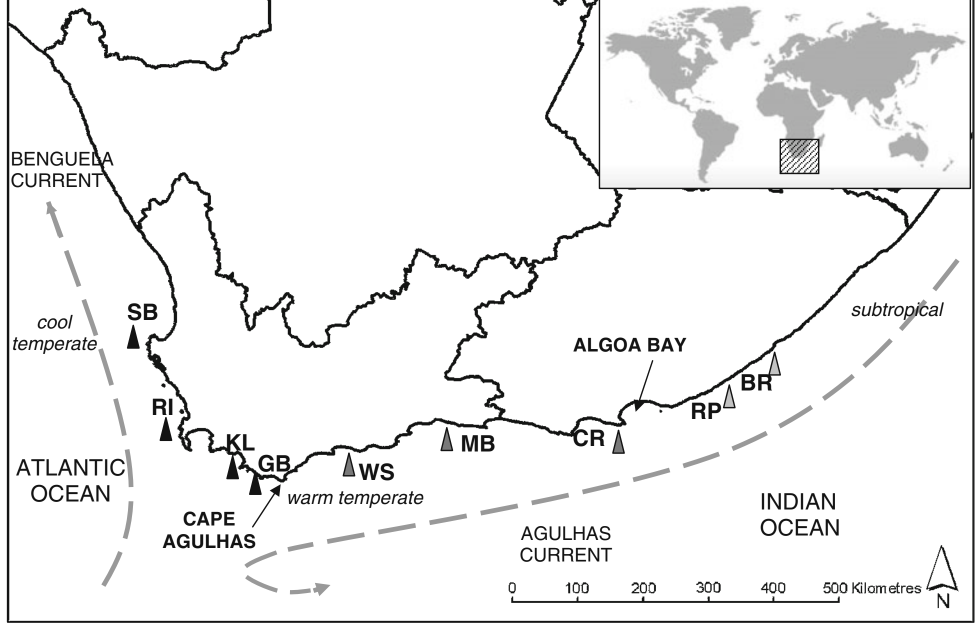


Fig. 1 Map showing the distribution of sample localities for *Haliotis midae* along the coast of South Africa. *1* Saldanha Bay (SB); *2* Robben Island (RI); *3* Kleinmond (KL); *4* Gansbaai (GB); *5* Witsand (WS); *6* Mossel Bay (MB); *7* Cape Recife (CR); *8* Riet Point (RP); *9* Black Rock (BR). The major oceanographic currents (Benguela and Agulhas) and the three major biogeographical provinces are indicated. *Arrows* denote the two potential areas of restricted gene flow tested for in this study

Agulhas and Algoa Bay (Norton 2005; Teske et al. 2006, 2007; von der Heyden et al. 2008) and several studies indicate varying levels of permeability of these barriers for different species (Tolley et al. 2005; Neethling et al. 2008). In addition, it is expected that the extension of the biogeographic regions have been affected by Plio-Pleistocene climatic conditions such as habitat contraction, latitudinal shift of water temperatures, and variations in currents and coastal topography (Flores et al.1999; Miller et al. 2005; Bard and Rickaby 2009).

Biogeographical boundaries are most often accompanied by discontinuities in the geographical distribution of genetic variation of inhabiting species. The methods at present most frequently applied are model-based clustering analysis that do not assume a priori grouping of individuals into populations. The latter has greatly advanced the ability for detecting genetic discontinuity (Pritchard et al. 2000; Latch et al. 2006) while methods including spatial information are considered most effective in estimating the number of genetic units in conservation biology (Chen et al. 2007; Segelbacher et al. 2010). Also, a combination of different types of molecular markers could aid in defining integrated management units because of their ability to reveal different levels of genetic structuring (e.g. Silva and Skibinski 2009). SNPs for example are often combined with microsatellites in population studies of marine organisms (Smith et al. 2007; Narum et al. 2008) because of their wider coverage of the genome, frequent association with genes and potential for large scale and cost effective genotyping (Seddon et al. 2005; Nielsen et al. 2009 and references therein). Also, SNPs associated with genes are more likely to be under the influence of selection and such loci are particularly useful in stock identification in fisheries and estimating the adaptive potential of metapopulations (Nielsen et al. 2009; Bonin et al. 2007). It is therefore important to apply appropriate tests of neutrality above and beyond measuring population structure (Rand 1996).

In this study, variation at eight microsatellite and 12 SNP loci was investigated in nine *H. midae* populations sampled throughout its known distribution range. The specific aims were (a) to identify genetically differentiated populations using frequentist analysis as well as Bayesian clustering methods and, more specifically, to test the hypothesis of genetic discontinuity across the Agulhas and other biogeographic boundaries along the South African coast, (b) to investigate past population processes that might influence the contemporary patterns of genetic variation, (c) to interpret present-day patterns of genetic variation in *H. midae* populations in terms of contemporary and historical evolutionary mechanisms, and (d) to apply these results to the formulation of specific conservation and management strategies for *H. midae*.

Materials and methods

Sampling collection and DNA extraction

A total of 428 tissue samples were collected from nine localities between 2000 and 2005 (Fig. 1). Sampling was performed within the species' natural distribution range extending from Saldanha Bay on the West coast to the mouth of the Kei River on the East coast (Table 1). Most of the collection occurred up to 1 km offshore and only animals >100 mm (4–5 years old) were considered for sampling. Genomic DNA was extracted from gill or muscle tissue using a standard cetyltrimethylammonium bromide (CTAB) method described by Saghai-Maroof et al. (1984). Approximately 0.1 g of gill or muscle tissue was placed in 700 µl of lysis buffer containing 0.5 mg/ml proteinase K and incubated overnight at 60°C. Following incubation, the samples were extracted with chloroform: isoamyl alcohol (24:1), ethanol precipitated and resuspended in distilled water. DNA integrity was verified by agarose gel electrophoresis and known standards. Original and diluted DNA stocks were stored at −20°C until further use. DNA samples from Kleinmond and Mossel Bay were kindly provided by R Bowie from the Department of Botany and Zoology at the University of Stellenbosch.

Microsatellite and SNP genotyping

Template DNA of all 428 samples was amplified at eight microsatellite loci (Table 2). Six of these loci, *HmD14*, *HmD36*, *HmD55*, *HmD59*, *HmA11* and *HmSP5,* were previously described by Bester et al. (2004). Polymerase chain reaction (PCR) amplification was performed in a GeneAmp 2700 thermocycler (Applied Biosystems) and each reaction contained 20–100 ng genomic DNA, 200 µM

Table 1 Sampling localities, sample size and their respective geographic coordinates

Sampling location	*n*	Geographic coordinates
West coast		
Saldanha (SD)	57	33°02′40.64″S; 17°56′00.53″E
Robben Island (RI)	48	33°48′17.12″S; 18°23′12.52″E
Kleinmond (KL)	48	34°20′53.37″S; 19°01′39.75″E
Gansbaai (GB)	48	34°35′04.00″S; 19°19′45.63″E
South coast		
Witsand (WS)	33	34°24′05.25″S; 20°51′13.19″E
Mossel Bay (MB)	54	34°10′31.81″S; 22°09′15.47″E
Cape Recife (CR)	48	34°02′15.98″S; 25°42′17.64″E
East coast		
Riet Point (RP)	53	33°31′29.31″S; 27°06′51.18″E
Black Rock (BR)	48	32°40′49.51″S; 28°23′54.08″E

Table 2 Summary of eight microsatellites studied in nine populations of *Haliotis midae*

Locus	Repeat	Primer sequences	Dye	T_a	N_a	H_O	H_E	Acc Nr
HmD14	$(CA)_{10}$	F TAAGGCAAGTGAATGTCTAG R ATTGCAAGAATCACAACTGC	NED	60	28	0.67	0.76	AY303333
HmD36	$(GTGA)_{14}$	F AGATCGAATGACATCAGCTTC R CATATAGCAAGCCTGAAACC	NED	60	42	0.43	0.89	AY303335
HmD55	$(GTGA)_{12}$	F ATCAAGATAAAACGAGGCG R ACCACTGTGAAAACGTCCA	VIC	60	25	0.68	0.8	AY303337
HmD59	$(CA)_{15}$	F TATACTGCCATTTCCGTCTG R TCTGTATTCTGGTCCTGTCG	FAM	60	26	0.78	0.84	AY303338
*Hm*A11	$(TCTG)_8$	F AGCTCAGAAAAGTGGTGTACG R TTACCTAGCTAAAGTTGACAACG	VIC	61	11	0.32	0.66	AY303341
HmSP5	$(AC)_{13}$	F TTCGGCAAGTGAATGTCTAG R ATGCGACACTTACTACACCG	FAM	60	29	0.63	0.74	AY303344
CmrHr2.15	$(CA)_{27}$	F TTTACATCGCATCGGCATTA R TACTTAACGTTGCCCTGCCT	NED	55	23	0.43	0.71	AF195956
HmAD102	$(ACTC)_{15}$	F ACATTGGGGTTCTCAATCA R TAACGGGACAATGAATAAACTA	VIC	60	39	0.53	0.82	DQ785747

T_a(°C) annealing temperature, H_O observed heterozygosity, H_E expected heterozygosity, N_a number of alleles

of each dNTP, 3–5 pmol of each primer, 2–3 mM $MgCl_2$ and 0.5 units of *Taq* DNA polymerase in a total volume of 10 µl. Thermal cycling conditions consisted either of a basic cycle profile of denaturation at 95°C, annealing at T_a and extension at 72°C or a touch-down method where the annealing temperature is lowered by 1°C with each consecutive cycle. Fluorescently labeled PCR products were separated on an Applied Biosystems 3100® Genetic Analyzer and allelic sizes were determined using the Genotyper® software 3.7.

Template DNA of 288 samples (32 samples per population) was also amplified for 12 SNP loci (Table 3) and all PCR reactions were performed as described in Bester et al. (2008). Following amplification, PCR products were assessed using gel electrophoresis and purified through SigmaSpin post-reaction purification columns. Automated sequencing was performed in both directions using the ABI PRISM BigDye Terminator 3.1 Cycle Sequencing kit and the 3100 Genetic Analyser. Alignment of sequences was carried out in the sequence alignment editor program BioEdit 7.0.9.0 and manually scored at each of the 12 SNP loci. The genotypes of each individual were also confirmed through manual inspection of the sequence chromatograms.

Table 3 Summary of 12 SNP loci genotyped in nine populations of *Haliotis midae*

EST clone	SNP ID	SNP	Primer sequences	T_a (°C)	H_O	H_E	Acc nr
1A1 Perlucin	*SNP-1*	A>C	F TTTCATGTTTTGCATCAAAC	57	0.33	0.31	EU135915
	SNP-2	G>A	R AAGAAGGAACTGTATGGCTG		0.87	0.49	
	SNP-3	G>T			0.33	0.32	
C12 Cellulase	*SNP-4*	T>C	F ATTTTTGTCGGTCACCTGGA	55	0.06	0.23	EU135914
	SNP-5	T>A	R GTAGGGCTTCCCAGAAGGAC		0.12	0.31	
3B4-1 *Ribosomal protein L8e*	*SNP-6*	C>T	F AAACATCTGCAACATTTAGG	57	0.51	0.48	EU135916
	SNP-7	A>T	R GACAGCAAAACAAACATCAG		0.44	0.48	
	SNP-8	C>T			0.45	0.49	
	SNP-9	A>G			0.48	0.49	
3B4-2 Ribosomal protein L8e	*SNP-10*	T>A	F TAAGAATCCACAAGTTGGTG	57	0.40	0.35	EU135916
	SNP-11	T>C	R ATGTATCATCACGGACAGG		0.45	0.43	
	SNP-12	T>A			0.32	0.31	

T_a(°C) annealing temperature, H_O observed heterozygosity, H_E expected heterozygosity

Data analysis

To assess genetic variation within and among sampling populations, basic estimates for genetic diversity such as number of alleles, allele frequencies, observed (H_O) and expected heterozygosity (H_E) and F_{is} (Weir and Cockerham 1984) were estimated using GENETIX 4.03 (Belkhir et al. 2000) and FSTAT 2.9.3 (Goudet 1995). Allelic richness was also calculated in FSTAT and compared among sampling regions using the Kruskal–Wallis test available in Analyse-it® for Excel. Deviations from Hardy–Weinberg equilibrium (HWE) were tested with the Markov chain Monte Carlo (MCMC) approximation of Fisher's exact test implemented in GENEPOP 4.0 (Raymond and Rousset 1995). MICRO-CHECKER 2.2.3 (Shipley 2003) was used to identify loci suspected of null alleles and scoring errors caused by stuttering and large allele dropout. Linkage disequilibrium was assessed according to Fisher's method available in GENEPOP. Deviation from neutrality was evaluated for all microsatellite and SNP loci using a F_{ST}-outlier method of Beaumont and Nichols (1996) implemented in LOSITAN (Antao et al. 2008). For all runs 50,000 simulations were generated using both mean and forced F_{ST} options assuming SMM.

To test for population differentiation among sampling locations, analysis was performed on all 20 markers combined. Separate datasets containing only microsatellites or SNPs and a combined data set in which the SNPs were reduced to haplotypes with PHASE 2.1 (Stephens and Donnelly 2003) were also investigated (only clustering results are shown in Supplementary materials 4 and 5). The following analytical methods were used: (1) Pairwise F_{ST} (2) Factorial correspondence analysis (3) Bayesian model-based clustering analysis and (4) Analysis of molecular variance.

The Arlequin 3.11 package (Schneider et al. 2000) was used to calculate pairwise F_{ST} values (Weir and Cockerham 1984), testing the null hypothesis of no differentiation by permuting the genotypes between populations (1,000 replicates). Significance levels were adjusted for multiple tests with Bonferroni correction (Rice 1989).

To view the distribution of genetic variation in a multidimensional format, allele frequency data was subjected to factorial correspondence analysis (FCA) available in GENETIX. FCA analysis was also conducted at the individual level to test whether geographical distribution could be related to the relative distribution of individual genotypes in the multivariate plot.

To infer the most likely number of genetically differentiated populations or clusters (K) present in the data, the Bayesian model-based clustering methods implemented in the software STRUCTURE 2.1 (Pritchard et al. 2000) and TESS 1.1 (Chen et al. 2007) were applied. For STRUCTURE, the log likelihood of the data [ln Pr(X/K)] was estimated for different values of K ($K = 1$–13) using the admixture model and correlated allele frequencies. Each run consisted of a burn-in of 10^5 cycles and 1×10^6 Markov Chain Monte Carlo (MCMC) iterations and repeated 10 times for each value of K. The uppermost level of genetic structuring was then estimated using the statistic delta K (ΔK), which corresponds to the second-order rate of change in likelihood values between successive K's (Evanno et al. 2005). Due to the a priori assumption regarding HWE within populations, six of the loci (*HmD36*; *HmAD102*, *Hr2.15*, SNP-2, SNP-4 and SNP-5) were excluded from the STRUCTURE analysis. Analysis was also repeated using the independent allele frequency model.

For the second clustering approach geographic coordinates were added and analysis of population structure performed by sequentially increasing the maximum number of clusters (K_{max}). The program TESS also allows for making prior assumptions about the level of interaction between populations such that when no spatial prior information is incorporated ($\Psi = 0$), the clustering model is similar to that of STRUCTURE. Using the admixture model and an interaction value of 0.6, 30 runs of 120,000 iterations and a burn-in of 50,000 were performed for $K_{max} = 2$–5. The 20% highest likelihood runs for each K_{max} were averaged in CLUMPP 1.1 (Jakobsson and Rosenberg 2007) and the program DISTRUCT (Rosenberg 2004) was used to graphically display the average membership coefficients of the clusters within each population. In addition, the Deviance Information Criterion (DIC; Spiegelhalter et al. 2002) was computed for each run.

Analysis of molecular variance (AMOVA) was carried out in ARLEQUIN using 1,000 permutations. The AMOVA was performed at three levels: within sampling populations, among sampling populations within groups and among groups. Genetic structure was tested under different grouping hypotheses (1) overall, (2) between two groups, populations west and east of Cape Agulhas (west vs east), and (3) between three groups SD/RI/KL/GB, WS/MB/CR and RP/BR (west vs south vs east).

Demographic parameters were examined using only microsatellite data. As effective population size (N_e) is often a key component in the conservation of threatened or endangered species, population specific N_e and long-term gene flow was estimated using the coalescent-based program MIGRATE 3.0.3 (Beerli and Felsenstein 2001). A Bayesian approach was used to estimate the posterior distribution of both effective population size ($\theta = 4N_e\mu$) and pair-wise migration rates ($M = m/\mu$, where m is the immigration rate and μ the mutation rate). Ten replicates of one long chain were run for 15,000 steps sampled, with increment of 20 and a burn-in of 10000, using an adaptive heating scheme of four temperatures (1.0, 1.5, 3.0 and 6.0).

Finally, recent changes in population size were examined using the method of Cornuet and Luikart (1996) implemented in BOTTLENECK 1.2.02 (Piry et al. 1999). This method tests for temporary HW heterozygote excess due to a bottleneck relative to that expected under mutation-drift equilibrium. Heterozygosity excess or deficiency was estimated for each locus separately and averaged across loci assuming three mutational models: (1) the stepwise mutational model (SMM), (2) the infinite allele model (IAM) and (3) the two phase mutational model (TPM) with 50% of mutations following SMM. The two-tailed Wilcoxon signed rank test was used to determine significance of the observed deviations.

Results

Genetic diversity, Hardy–Weinberg equilibrium, linkage disequilibrium and neutrality testing

Moderate to high levels of genetic diversity characterized all nine *H. midae* populations, which are comparable to what has been reported for other *Haliotis* species (Tang et al. 2004; Imron et al. 2007). The microsatellite loci exhibited moderate to relatively high levels of polymorphism with the total number of alleles per locus ranging from 12 to 43. Observed and expected heterozygosity, averaged over all populations, varied from 0.35 to 0.848 and 0.583 to 0.939 respectively while F_{IS} values were significant for four of the loci ($0.001 < P < 0.01$) (Supplementary material 1). The average number of alleles did not differ significantly between populations but when three groups (W–S–E) were considered, three microsatellite loci showed higher allelic richness in the South coast, and the remaining loci showed either similar diversity across regions or slightly lower diversity in the East. When considering two groups (W–E), two loci showed significantly higher diversity in the East and the remaining loci showed a similar allelic richness (Supplementary material 2). Global tests (P-values over all loci and populations) for Hardy–Weinberg equilibrium were highly significant ($P < 0.001$) and only four loci (*HmD14*, *HmD55*, *HmD59* and *HmSP5*) were in equilibrium over all populations. MICRO-CHECKER further revealed that null allele frequencies were highly significant ($P < 0.001$) for loci *HmD36*, *HmAD102* and *Hr2.15*. Ideally, primer redesign would have confirmed and corrected for null alleles present in this study, but was not considered as the highly polymorphic nature of this species demanded a relatively high number of alternative primers to be tested. Where necessary, data analysis was performed excluding loci with putative null alleles.

For the SNPs, observed and expected heterozygosity, averaged over all populations, ranged from 0.121 to 0.870 and 0.232 to 0.489 respectively while the minor allele frequency ranged from 0.143 to 0.489 (Supplementary material 3). The exact test performed with GENEPOP showed that three of the SNP loci (SNP-2; SNP-4 and SNP-5) deviated significantly from Hardy–Weinberg equilibrium ($P < 0.05$) while none of the populations were in equilibrium over all loci. Significant linkage disequilibrium ($P < 0.05$) was observed only for pairs of SNPs originating from the same EST sequence. The latter further increased expectations of diminished statistical power compared to the microsatellites but results of Morin et al. (2009) demonstrated that although linked loci reduce the coverage of the genome, the statistical power of SNPs for population structure can actually be increased by including multiple SNPs within loci and inferring haplotypes rather than using only unlinked loci. In this study, the very small number of haplotypes inferred with PHASE was unlikely to have resulted in a significant gain in statistical power. No linkage disequilibrium was detected between microsatellite and SNP loci. With LOSITAN, based on the 50,000 simulations, the loci *HmAD102*, SNP4 and SNP5 were identified as candidates for positive selection with P-value (simFst < obsFst) >0.99.

Population structure

Here results are described for all markers combined although comparisons are made with the separate data sets where applicable. Pairwise F_{ST} values were relatively low ranging from −0.0057 to 0.0339 and the global test over all populations was not significant ($F_{ST} = 0.0168$, $P > 0.05$). Five F_{ST} pairwise values were significant after sequential Bonferroni adjustment (Table 4), which involved either Gansbaai or Witsand, the two populations situated on either side of the hypothetical Cape Agulhas barrier. Factorial correspondence analysis (FCA) allowed a three-dimensional view of the genetic relationships among populations with the first three factors explaining 46.71% of the population differentiation. Although separation of the West coast populations from the rest could be viewed along the first factor (Fig. 2), the distribution of the populations did not relate to their geographical positioning along the coastline. FCA analysis of the individual genotypes did show substantial overlapping of West, South and East coast individuals, but the distribution along the first axis mostly agreed with a west-east coast partitioning.

The STRUCTURE analysis based on the combined dataset suggested the existence of either two or three distinct clusters, depending on the allele frequency model used. Under the correlated allele frequency model, the log-likelihood values were highly variable between different estimates of K; the ad hoc statistic ΔK analysis (Evanno et al. 2005) showed the highest peak at $K = 3$ (Fig. 3).

Table 4 Pairwise F_{ST} (θ) values between populations of *Haliotis midae*

	SD	RI	KL	GB	WS	MB	CR	RP	BR
SD	0								
RI	−0.0014	0							
KL	0.0066	0.0017	0						
GB	0.0107*	0.0104	0.0154*	0					
WS	0.0138	0.0228*	**0.0304***	**0.0331***	0				
MB	0.0076	0.0132	0.0249*	**0.0307***	−0.0057	0			
CR	0.0073	0.0138	0.0239*	**0.0311***	0.0111*	0.0026	0		
RP	0.0071	0.0135	0.0152*	**0.0339***	0.0085*	0.0067	0.0052	0	
BR	0.0102	0.0122	0.0140*	0.0221	0.0160	0.0111	0.0155*	0.0121	0

Significant values after Bonferroni adjustment are indicated in bold

* Significance at 0.05 level

For abbreviations of populations see Table 1

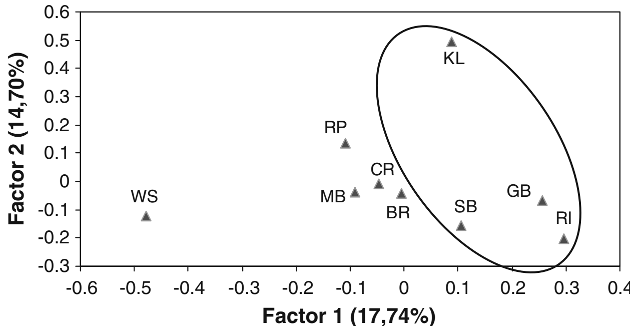


Fig. 2 Factorial correspondence analysis plot of nine populations of *Haliotis midae* along factors 1 and 2 where the West coast populations are encircled (for abbreviations see Table 1)

When the combined data set was tested under the independent allele frequency model however, the log-likelihood values varied little for values of $K > 3$ and only two significant clusters were distinguished based on ΔK. The latter was also observed for the separate data sets (Supplementary material 4) and consequently a two-cluster model was used to assign individuals to hypothetical West and East coast clusters. The proportion of membership (Q) showed high levels of admixture ($Q > 0.25$ or $Q < 0.75$) at the individual as well as the population level and none of the sampling locations was strongly associated with either of the clusters. The subtle break between the western and eastern populations was only evident from the population summary plot based on 20% of the highest likelihood runs permutated in CLUMPP.

The clustering pattern (averaged over populations) obtained with TESS was also consistent with either a two or three population model. Initially, runs with different values of the interaction parameter revealed consistency between $\Psi = 0.5$ and $\Psi = 0.6$ while higher values of Ψ mostly resulted in single clusters. More than 50% of the individual runs with $K_{max} = 4$ and $K_{max} = 5$ resulted in $K < 4$ which suggested that the most likely distribution of the populations were into two or three clusters. The permutation effect of CLUMPP resulted in a bidirectional assignment of clusters such that clinal variation of allele frequency was evident along a longitudinal gradient for all values of K_{max} tested (Fig. 4). A west-east coast cline was most evident for the microsatellite only data where the central population of Witsand consistently showed admixture of the primary clusters for all K_{max} values (Supplementary material 5). The latter was also consistent with a secondary contact zone between western and eastern populations. It should be noted that the DIC values were not used to test the applicability of a clinal model to our data but did show the steepest decrease from $K_{max} = 2$ to $K_{max} = 3$, similarly to what was found for killifish where a contact zone best explained the data using spatial analysis together with DIC evaluation (Durand et al. 2009).

The AMOVA analysis revealed weak but significant structure between the three coastal regions (Table 5). Significant values were obtained when the grouping hypotheses 'west vs east' ($F_{CT} = 0.011$, $P = 0.013$, 1.13% of the variation) and 'west vs south vs east' ($F_{CT} = 0.011$, $P = 0.001$, 1.06% of the variation) were tested. For both grouping analysis, most of the variance was distributed between individuals within populations (98.09%; 98.30%) while very little variance was distributed among populations within groups (0.78%; 0.65%).

Demographic analysis

The Bayesian estimates of theta (θ) computed in MIGRATE gave an indirect estimation of long-term effective population size per population utilizing microsatellite data only. Except for the most eastern population of Black Rock, the average θ values varied very little between populations and comparable values within the

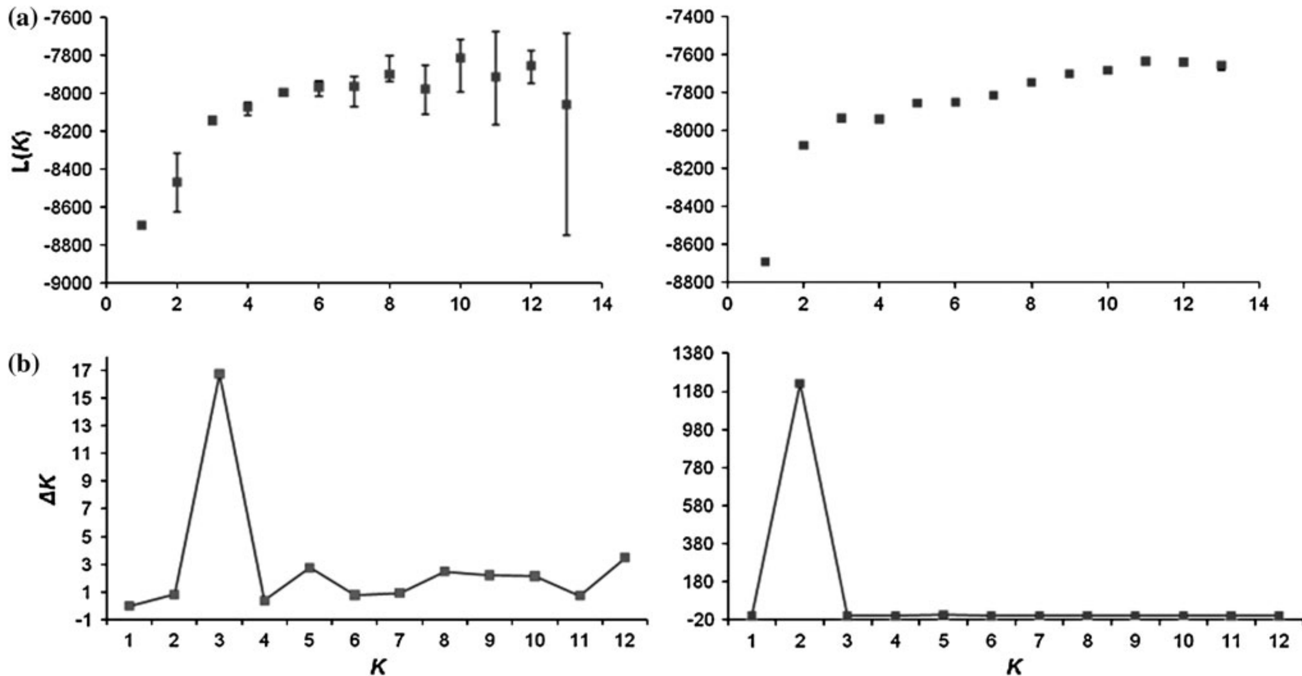


Fig. 3 Clustering analysis as performed in STRUCTURE showing **a** The posterior probability of the data [L(K)] over 10 runs assuming $K = 1$–13 and **b** ΔK as a function of K following Evanno et al. (2005) using the correlated (*left*) and independent (*right*) allele frequency model

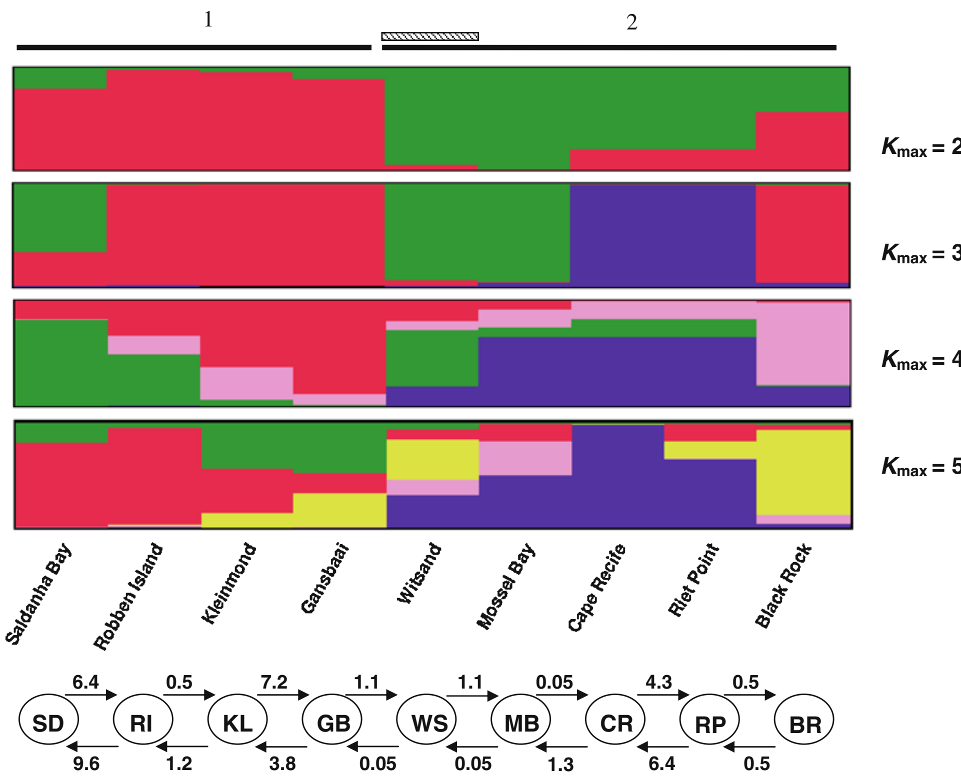


Fig. 4 Estimated cluster configuration of nine *Haliotis midae* populations calculated by TESS ($\Psi = 0.6$ and $2 \leq K_{\max} \leq 5$), permutated by CLUMPP and visualised by DISTRUCT. The histograms shown here are for the combined data set. The proposed West (1) and East (2) coast groups and the admixture zone are indicated with *black and streaked horizontal bars* respectively. Relative directionality of gene flow between populations and migration rates as estimated in MIGRATE are denoted at the end of the figure

different coastal regions (West, South and East coast) indicated stable long-term population sizes (Supplementary material 1). Bayesian estimates of long term migration rates were considered only between pairs of adjacent populations assuming a stepping-stone model of migration (Fig. 4). The most outstanding result is the long-term restriction to gene flow among the populations on the South coast, whereas the West and East coast neighboring

Table 5 Hierarchical AMOVA among nine *Haliotis midae* populations under two grouping hypothesis

Source of variation	df	Sum of squares	Variance	Variation (%)	Fixation indices	*P*-value
'West vs East'						
Among groups	1	21.618	0.052	1.13	0.011	0.013
Among populations within groups	7	47.566	0.036	0.78	0.008	0.015
Within populations	567	2555.797	4.508	98.09	0.019	<0.0001
'West vs South vs East'						
Among groups	1	30.747	0.049	1.06	0.011	<0.001
Among populations within groups	7	38.438	0.030	0.65	0.007	0.025
Within populations	567	2555.797	4.507	98.30	0.017	<0.0001

df degree of freedom

populations display higher migration rates (with 95% CI not encompassing 0). Restricted gene flow between RI-KL and RP-BR could indicate a minor historical restriction to gene flow, which also seems apparent at higher levels of K with Bayesian clustering analysis ($K = 5$, Fig. 4a–c). Similar results were obtained when (i) excluding loci with suspected null alleles or (ii) excluding locus *HmAD102* under positive selection.

For all the microsatellite loci tested, BOTTLENECK showed an imbalance between the two heterozygosity estimations. Estimates expected under HWE were much higher than that for mutation-drift equilibrium, indicating heterozygote deficiency. Averaged over loci, the Wilcoxon signed rank test indicated significant departure from mutation-drift equilibrium under the TPM model (*P* one tail for H deficiency = 0.01563) and the SMM model (P one tail for H deficiency = 0.00781) and equilibrium under the IAM. These results were indicative of demographic expansion.

Discussion

Population structure: contemporary and historical signals

Population structure in marine species has been ascribed to the interplay between contemporaneous physical and ecological forces; it is essentially the consequence of geographical distance, ocean dynamics, environmental transitions and life-history changes (Hemmer-Hansen et al. 2007). The most prominent present-day feature responsible for limiting gene flow in South African abalone appears to be the physical barriers originating from the ocean currents along the South African coast. Microsatellite and SNP genotypes analyzed supported two subtle but significant discontinuities in the genetic composition of populations along the South African coast: the most prominent break is located around Gansbaai-Witsand in the region of Cape Agulhas and a second break within the South/East coast biogeographical boundary (Cape Recife-Riet Point). The West and East coast groups seem to transition in an admixture zone, which might coincide with the "third group" found in the AMOVA and STRUCTURE analysis. It seems likely that the barrier created by the retroflection of the Agulhas current (Dijkstra and de Ruijter 2001), may prevent larvae from dispersing further or act as a barrier to dispersal of larvae from west to east. Recently, Zardi et al. (2007) found unexpected structure between indigenous mussel populations from the West and East coast of South Africa and suggested that together with historical and ecological factors, the Agulhas current could be the main feature responsible for the genetic disjunction. Similarly, the thermal front at Port Elizabeth could affect dispersal patterns along the east coast of South Africa although this barrier is not as strongly supported by the population structure analysis performed here for *H. midae*. The increasing evidence for association of intraspecific lineages with the three major marine biogeographic provinces of South Africa (Ridgway et al. 1998; Teske et al. 2006, 2007, 2008; von der Heyden et al. 2008), supports the idea that region-specific oceanographic processes play a significant role in the structuring of species along the coast. Species distributed along several coastal biogeographic provinces, are subjected to diverse physical and biological environments, leading to local adaptation and genetic divergence. The broad morphological similarity between West and East coast samples suggests that *H. midae* is well adapted to a wide range of environmental factors, including temperature. However, the possible contribution of differential selective forces to the apparent genetic discontinuity can only be excluded based on experiments specifically designed to detect adaptive divergence. The putative selected loci identified in this study are interpreted with caution as Beaumont and Nichols (1996) recommend that more than 20 loci be used for their method to be effective in detecting outlier loci. Additional loci and/or analysis methods should be incorporated to confirm results

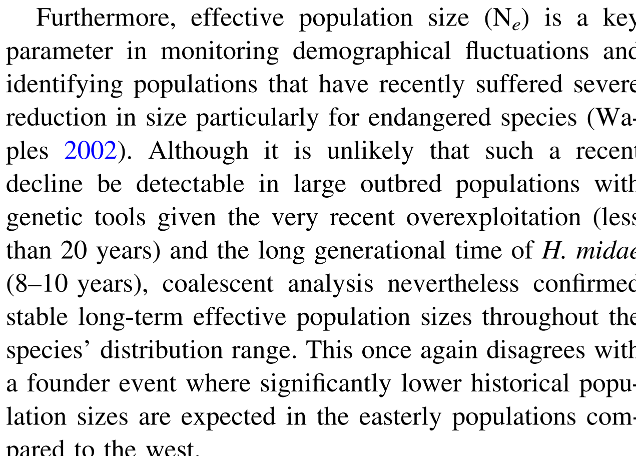
regarding neutrality of loci. A study is currently in progress to investigate the underlying molecular genetic processes of divergent selection between West and East coast abalone using a population genomic approach (Clint Rhode, pers. comm.).

Nonetheless, coalescent analysis of long-term migration rate implied restriction to gene flow on the South coast. The hypothesis of a secondary contact zone or admixture zone was in addition supported by the observation of allelic richness which for three loci is highest for the South coast populations. From a historical perspective therefore, associated consequences of glaciations such as sea level changes (Yokoyama et al. 2000; Miller et al. 2005), latitudinal shifts in water temperature (Cutler et al. 2003; Bard and Rickaby 2009), changes in coastal topography and currents (Flores et al. 1999) could have been the underlying shapers of population structuring in *H. midae* today. For instance, it is possible that abalone along the southernmost tip of Africa were not able to survive or adjust to the much lower water temperatures during inter-glacial periods, giving rise to isolated refugia between the West and East coast of South Africa.

Considering all factors described above it is more likely that the biogeographic break at Cape Agulhas acts as the main barrier to gene flow and that the structure observed today could at the same time reflect formerly isolated populations that came into secondary contact following range expansion in both directions, a feature commonly observed worldwide.

Demographic history

Population size expansion and possibly a re-colonization event from the western and eastern coastal waters followed by range expansion in both directions characterize the South African abalone. Population expansion postdating the Last Glacial Maxima (~20,000 ybp) has also been documented for other South African marine fauna such as spiny lobster *Palinurus gilchristi* (Tolley et al. 2005), *Palinurus delagoae* (Gopal et al. 2006), Cape hake *Merluccius merluccius* (von der Heyden et al. 2007), *Caffrogobius caffer* (Neethling et al. 2008) and Cape fur seal *Arctocephalus pusillus pusillus* (Matthee et al. 2006). It demonstrates the profound influence that glaciations had on population structure and gene flow even on Southern Hemisphere species. A break in gene flow and range expansion has previously been identified in *H. midae* by Evans et al. (2004) who proposed a westward founder event based on mtDNA and limited microsatellite data. An alternative, more plausible hypothesis presented here is a secondary contact between two historically isolated units, evidenced mainly from the spatial Bayesian clustering analyses and coalescent-based long-term migration rates.



Furthermore, effective population size (N_e) is a key parameter in monitoring demographical fluctuations and identifying populations that have recently suffered severe reduction in size particularly for endangered species (Waples 2002). Although it is unlikely that such a recent decline be detectable in large outbred populations with genetic tools given the very recent overexploitation (less than 20 years) and the long generational time of *H. midae* (8–10 years), coalescent analysis nevertheless confirmed stable long-term effective population sizes throughout the species' distribution range. This once again disagrees with a founder event where significantly lower historical population sizes are expected in the easterly populations compared to the west.

In summary, demographic data provided no evidence for recent population decline but rather suggest that *H. midae* experienced population size expansion in the past. Secondary contact following historical isolation along with present day restriction to gene flow is probably more relevant to contemporary populations than the founder event previously proposed for *H. midae*.

Conservation and management implications

The identification of stock structure is especially important in marine species that have recently shown a dramatic decline in numbers or have become increasingly threatened by human and other interferences e.g. corals (Van Oppen and Gates 2006); loggerhead turtles, *Caretta caretta* (Bowen et al. 2005); sockeye salmon (Kozfkay et al. 2008) and white abalone, *H. sorenseni* (Gruenthal and Burton 2005). Populations are typically recognised as separate management units when genetic differentiation is found (Moritz 1994) and/or adaptive divergence has occurred between geographically separated groups (Crandall et al. 2000).

Until now, management of wild *H. midae* entirely relied on measures such as minimum legal size (MLS), annual quotas, total allowable catch (TAC), closed seasons and marine reserves (Tarr 2000). With increasing pressure on the resource and the knowledge that recovery of abalone fisheries world-wide has proven to be complex (Tegner 2000), an integration of genetic and ecological management is paramount to ensure survival of remaining stocks. This study shows significant restriction to gene flow around Cape Agulhas while a secondary barrier in the Algoa Bay region seems less tight. It is therefore necessary to develop and incorporate management measures that take the population dynamics as well as demographic analysis into account. The minimal precautionary measures would be to regard populations west and east of Cape Agulhas as separate stocks and ideally these units should be managed separately. On the other hand, the transition zone and

secondary break along the east coast should also be regarded as crucial information and considering only two reproductive stocks could in fact compromise the adaptive diversity and therefore evolutionary potential of the species (Crandall et al. 2000; Fraser and Bernatchez 2001). A realistic approach would therefore be to treat the species as populations connected by various degrees of gene flow while aiming to preserve the natural connectivity between them. Especially with regards to commercial abalone fisheries and intended recovery programs, intentional seeding of cultured abalone should only be performed with animals that match the genetic profile of the immediate surrounding wild gene-pool. For *H. midae,* the minimum measures would once again entail separation of the West and East coast stocks while taking into consideration a transitional zone east of Cape Agulhas. Most importantly, the transport of larvae or juveniles between farms situated in different coastal regions should be regulated whilst minimizing accidental release of commercial stock into the wild. Genetic diversity within commercial stocks should also be evaluated on a regular basis to avoid unintentional contamination of the wild stocks with animals from a potentially different adaptive background.

The role of marine protected areas (MPAs) in the future conservation of South African abalone remains just as important (Attwood et al. 1997; Palumbi 2003) and the existing MPAs intended to conserve ecosystems should include an evaluation for their use as conservation sites for *H. midae*. It is expected that MPAs that take an ecosystem approach by protecting key species such as kelp and sea urchins will indirectly benefit abalone. MPAs were initially intended to combat the loss of biodiversity, be it on an ecosystem or species-specific level (von der Heyden 2009), but have proven invaluable for the sustainability of natural goods, services and functions. It is therefore clear that the inclusion of genetic and environmental information in commercial fisheries, recovery or enhancement programs are essential to conserve the South African abalone. Crandall et al. (2000) introduced the concept of exchangeability where populations are defined as conservation units only when the hypothesis of genetic as well as ecological exchangeability on recent and historical time scales can be rejected. In the future, experiments of ecological exchangeability in which adaptive divergence in relevant traits is tested, could assist in a more integrative designation of conservation units for *H. midae* but for now the genetic information provided here should be regarded as crucial for sustainable management of *H. midae* stocks.

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