marine ecology
an evolutionary perspective

Marine Ecology. ISSN 0173-9565

ORIGINAL ARTICLE

Genetic diversity and population structure of the size-selectively harvested owl limpet, *Lottia gigantea*

Phillip B. Fenberg[1], Michael E. Hellberg[2], Lynne Mullen[3] & Kaustuv Roy[4]

1 Department of Palaeontology, The Natural History Museum, London, UK
2 Department of Biological Sciences, Louisiana State University, Baton Rouge, LA, USA
3 Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA, USA
4 Section of Ecology, Behavior, and Evolution, University of California, San Diego, La Jolla, CA, USA

Keywords
Lottia gigantea; microsatellites; size-selective harvesting.

Correspondence
P.B. Fenberg, Department of Palaeontology, The Natural History Museum, Cromwell Road, London SW7 5BD, UK.
E-mail: p.fenberg@nhm.ac.uk, PFenberg@gmail.com

Accepted: 2 May 2010

doi:10.1111/j.1439-0485.2010.00386.x

Abstract

Size-selective harvesting can elicit a genetic response in target species through changes in population genetic subdivision, genetic diversity and selective regimes. While harvest-induced genetic change has been documented in some commercially important species through the use of historic samples, many commonly harvested species, such as coastal molluscs, lack historic samples and information on potential harvest induced genetic change. In this study, we have genotyped six microsatellite markers from populations across much of the California mainland range of the size-selectively harvested owl limpet (*Lottia gigantea*) to explore the genetic structure and diversity of this species. We found no significant genetic structure or differences in genetic diversity among populations of *L. gigantea*. Our results suggest high gene flow among populations and that differences in life history, demography, and body size previously observed between protected and exploited populations is largely due to phenotypic plasticity. From a conservation perspective, if proper actions are taken to curb harvesting, then exploited populations should be able to return to their pre-impact state given sufficient time.

Problem

Size-selective harvesting is one of the most widespread anthropogenic impacts on marine species. The loss of larger (older) individuals due to such harvesting has numerous cascading effects on the biology of affected species and populations, including changes in life history, demography, reproductive output, and even ecological interactions (Fenberg & Roy 2008). Size-selective harvesting has been shown to elicit rapid microevolutionary responses in some wild populations (Coltman *et al.* 2003; Grift *et al.* 2003; Olsen *et al.* 2004; Kuparinen & Merila 2007; Nussle *et al.* 2009) and intense harvest-induced mortality (regardless of selectivity) over multiple generations should result in genetic changes (Allendorf *et al.* 2008). However, for the vast majority of affected species, we do not know whether observed changes have a genetic basis, represent phenotypic plasticity, or result from a combination of both (Kuparinen & Merila 2007; Allendorf *et al.* 2008; Fenberg & Roy 2008).

The most powerful way to detect harvest-induced genetic changes is to analyze samples collected over an extended period of time as a species is exploited (Coltman *et al.* 2003; Kuparinen & Merila 2007; Allendorf *et al.* 2008). This approach has produced mixed results for harvested fish species, with some such studies showing a change in genetic diversity or structure over time (Smith *et al.* 1991; Hauser *et al.* 2002; Heath *et al.* 2002; Hutchinson *et al.* 2003) while others reveal no significant trends (Nielsen *et al.* 1999; Ruzzante *et al.* 2001; Chevolot

et al. 2008). Such historical analyses are, however, not possible for the majority of harvested species due to the lack of archived samples from the past.

A comparison of genetic variation between populations of a target species from well-protected marine reserves with those from exploited areas is a valuable alternative to detecting harvest-induced genetic change when historic samples are not available. However, a prerequisite for such studies is the proper establishment of population genetic substructure of the target species (Coltman *et al.* 2003; Allendorf *et al.* 2008). Comparisons between protected and exploited populations of a panmictic species will provide little evidence of harvest-induced genetic change without historical data. On the other hand, if genetic differences exist, then comparisons of protected and exploited sites can provide information about an intended function of protection, the preservation of intraspecific genetic diversity (Trexler & Travis 2000). For example, allelic richness for a harvested fish species (*Diplodus sargus*) is significantly higher at protected areas compared with non-protected areas (Perez-Ruzafa *et al.* 2006). Interestingly, depleted populations of the Tasmanian black-lip abalone (*Haliotis rubra*) have higher levels of genetic diversity than do healthy populations (Miller *et al.* 2009). This is hypothesized to be due to a higher ratio of migrants to self-recruiting larvae at depleted populations relative to healthy populations (Miller *et al.* 2009). Thus information about patterns of gene flow and population connectivities of harvested species is necessary for better understanding the long-term consequences of harvest-induced mortality.

Intertidal and subtidal species of molluscs represent a large portion of species currently known to be affected by size-selective harvesting (Fenberg & Roy 2008). Humans have exploited coastal molluscs (clams, limpets, abalone, and other gastropods) as a food source since prehistoric times (Jerardino *et al.* 1992; Siegfried 1994; Mannino & Thomas 2002), and for many species subsistence harvesting in the past has given way to widespread commercial harvesting. However, there is a general lack of data on the effects of harvesting (commercial, recreational or subsistence) on the genetic population structures of shallow-water molluscan species. Even along the coast of California, where many species of molluscs are routinely harvested (Murray *et al.* 1999), information about genetic structure is only available for a few species (Gruenthal *et al.* 2007; Addison *et al.* 2008; Gruenthal & Burton 2008).

In this study, we examine the genetic diversity and population structure of one of the most widely exploited molluscs in California, the owl limpet *Lottia gigantea*. *Lottia gigantea* is an intertidal gastropod that can reach a maximum length greater than 100 mm, making it the largest patellogastropod (limpet) in North America. The geographic range of this species extends from Baja California (26.1 °N) to northern California (39.4 °N). Individuals undergo a single spawning event in the winter months, typically in January or February (Daly 1975). Larvae are pelagic and although the exact larval duration of this species remains unknown, laboratory studies of other Northeastern Pacific *Lottia* species (*Lottia digitalis* and *Lottia asmi*) suggest that metamorphic competence (at 13 °C) occurs around 5 days after fertilization (Kay & Emlet 2002). However, even with a short pelagic phase, larvae within the north-flowing coastal Davidson Current during the winter could potentially be transported long distances, upwards of 350 km (Glickman 1999; Gruenthal *et al.* 2007). The potential for high rates of dispersal between *L. gigantea* populations is thus substantial. On the other hand, there are physical and biological reasons to think that local retention of larvae is also possible. For example, rocky intertidal habitats in the middle portion of the geographic range of *L. gigantea* (Southern California) are often separated by long stretches of unsuitable sandy habitats and provincial and phylogeographic boundaries (*e.g.* Point Conception and Monterey Bay), as well as topographical headlands, which can also limit larval dispersal (Burton 1998; Dawson 2001; Wares *et al.* 2001; Banks *et al.* 2007). Perhaps more importantly, human exploitation of this species has the potential to affect the genetic makeup of local populations.

Decades of size-selective harvesting have reduced the body sizes of most California mainland populations of *L. gigantea,* with the exception of those living in well protected reserves and along isolated stretches of coastline (Pombo & Escofet 1996; Lindberg *et al.* 1998; Roy *et al.* 2003; Sagarin *et al.* 2007). Exploited and protected populations of this species differ in several ways. On average, individuals of *L. gigantea* from protected populations are larger, grow faster, change sex at a larger size (*L. gigantea* is a protandric hermaphrodite), are less abundant, and have different ecological interactions with the surrounding community compared with those from exploited populations (Pombo & Escofet 1996; Lindberg *et al.* 1998; Roy *et al.* 2003; Sagarin *et al.* 2007; Fenberg 2008). Besides humans, American black oystercatchers (*Haematopus bachmani*) also eat *L. gigantea*, yet they do not hunt for the large individuals, are sensitive to human encroachment, and do not regularly occur at sites frequented by humans (Lindberg *et al.* 1998).

Exploited populations have significantly lower biomass and total reproductive output compared to protected areas (Fenberg 2008). Such reductions in reproductive output can decrease the number of migrants among local populations and potentially result in a loss of genetic variation (Allendorf *et al.* 2008). The primary goal of this study is to quantify patterns of genetic connectivity among *L. gigantea* populations along the California coast using

microsatellite polymorphisms. In addition, we explore whether populations from exploited areas have significantly different levels of genetic diversity compared to those from protected marine reserves.

Material and Methods

Sample sites

We collected 218 *Lottia gigantea* individuals between 2004 and 2006 at eight rocky intertidal field sites spanning most of the California mainland portion of the geographic range of this species, from 32.66 °N to 38.18 °N (Fig. 1). We stored individuals in 95% ethanol (at 4 °C) and extracted DNA using a commercially available genomic DNA extraction kit (Bioneer Inc.).

We consider three of our field sites to be protected from substantial harvest pressure as a result of either proper law enforcement (Cabrillo National Monument) or from restricted public access (Vandenberg Air Force Base and Pebble Beach Golf Course). Two sites are considered exploited based on our personal observations of harvesting (La Jolla) and communication with reserve managers (Don Canestro at Kenneth S. Norris Rancho Marino Reserve) and another site on the Palos Verdes peninsula (PV) was placed in the exploited category based on observations that individuals collected at this location in the past were significantly larger than they are today (Roy *et al.* 2003). A historical decline in body size in Southern California has been attributed to size-selective harvest pressure (Roy *et al.* 2003; Fenberg 2008). Each site listed above was further confirmed to be either exploited or protected based on a previous study documenting the extent of size-selective harvesting of this species across much of its geographic range (Sagarin *et al.* 2007). The remaining two field sites (Pt. Reyes National Seashore and Bolinas) are near the northern range limit of *L. gigantea*, where factors such as naturally reduced abundances and a lack of small (young) individuals (compared to more southerly populations) make it difficult to asses the impact humans have had on these local populations (Fenberg 2008). We have therefore not placed these two field sites into a vulnerability category (protected or exploited); we do, however, use them for the analysis of overall population structure (see below). *Lottia gigantea* are very rare north of San Francisco CA (37.8 °N) (see Fenberg 2008), therefore our sample sizes at Bolinas (37.89 °N) and Pt. Reyes (38.18 °N) were reduced to limit damage to these populations. Increasing our sample sizes by removing a small piece of foot tissue is not recommended in the case of *L. gigantea* because personal observations suggest a high mortality rate is associated with this practice. In addition, our scientific collecting permits limited sample sizes to no more than 25–30 individuals at the protected sites.

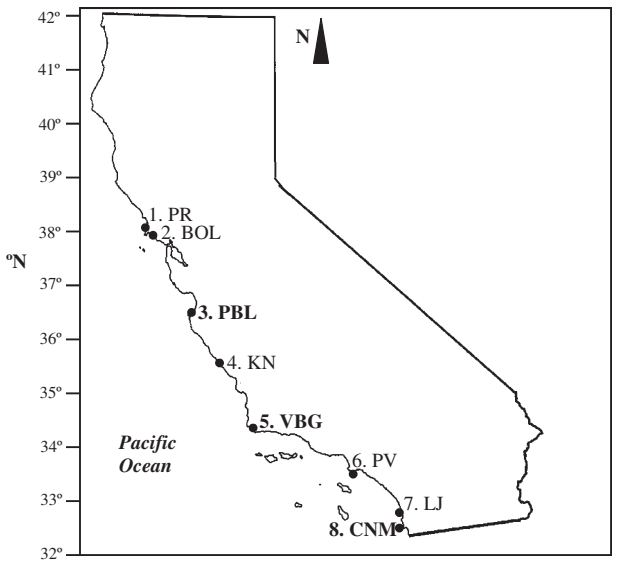

Fig. 1. Map of California collection sites for *Lottia gigantea*. Protected populations are in bold. PR: Pt. Reyes (38.18 °N); BOL: Bolinas (37.89 °N); PBL: Pebble Beach (36.56 °N); KN: Ken Norris UC Reserve (35.52 °N); VBG: Vandenberg (34.73 °N); PV: Palos Verdes (33.7 °N); LJ: La Jolla (32.82 °N); CNM: Cabrillo National Monument (32.66 °N).

Genotyping and statistical analyses

With the help of researchers at the Joint Genome Institute (JGI) (especially Jarrod Chapman), we screened the *Lottia gigantea* genome, which is currently being sequenced by JGI, for tri-nucleotide microsatellite markers with eight or more repeat units. This involved creating a long list of potential tri-nucleotide repeats (*e.g.* AAC, AAG, AGA, ATG, ATC, *etc.*) and requesting that JGI screen for their presence in the genome with the inclusion of 250-base-pair flanking regions of the repeat sequence each time it was present in the genome.

In all, we developed primers for 20 loci using PRIMER3 V. 0.4.0 (Rozen & Skaltesky 2000). Our criteria for keeping a locus for further analysis were based on the ease of amplification and our ability consistently to detect no more than two bands per individual. In all, we genotyped six polymorphic loci for each of our sampled field sites. PCR amplification for these six loci was performed using fluorescently labeled primers (Table 1) with an annealing temperature of 50 °C. Product sizes were visualized with an automated sequencer (ABI 3100). An internal size standard (400 HD ROX, Applied Biosystems) allowed for accurate sizing and the electropherograms were analyzed using GENEMAPPER V. X software (Applied Biosystems). Alleles were scored by PCR product size and converted to repeat number by subtracting the

Table 1. Primer sequences and product sizes for six *Lottia gigantea* microsatellite loci.

Locus	Left primer	Right primer	~Size (bp)
Lg1 $(ATC)_n$	tgttcttggcatcatcaaactt	gcatcacaaaggtgcaaaga	263
Lg2 $(AAC)_n$	ttacaaccgaacagctcagg	gttggtgctgttgttgatgg	354
Lg3 $(AAC)_n$	caaagcgctagccctaaaac	ctgcggctgatttcttcttc	299
Lg4 $(TGA)_n$	aacatgaatgatttaggggaag	gctgtctttgtttttaaccgtgt	212
Lg5 $(AAC)_n$	gccgatattggttgattagaca	tatgctggttgttgcattgg	223
Lg6 $(GTT)_n$	acgacacggcatgtgtctta	gggatttagcgttttgcgta	259

size of the flanking regions. We calculated the number of alleles and the expected and observed heterozygosity values across field sites for each locus using the program GENEPOP v. 3.4 (Raymond & Rousset 1995, 2003; Table 2). In addition, we account for reduced sample sizes at northern sites (see above) using a method employed by the program FSTAT (Goudet 1995) that allows us to estimate the mean allelic richness values per locus and field site based on the minimum sample size.

Samples were tested for deviations from Hardy–Weinberg equilibrium (HWE) across sites (multilocus) and by locus (multisite). Global and pairwise exact tests of genotypic differentiation were performed. In addition, we tested for evidence of linkage disequilibrium across all pairwise comparisons of loci. We performed the above statistical tests using GENEPOP with the following Markov chain parameters: 10,000 dememorization steps, 1000 batches, 10,000 permutations per batch.

Genetic differentiation between populations was further tested using F_{st} (Weir & Cockerham 1984) and the microsatellite analogue ρ_{st} between all pairwise field site possibilities using GENEPOP. ρ_{st} takes into account allele size assuming a stepwise mutation model (Michalakis & Excoffier 1996; Rousset 1996). In addition, we also calculated a standardized measure of F_{st} (F'_{st}), which accounts for low F_{st} values due to high within-population variation often found with microsatellites (Hedrick 2005). Levels of significance were based on sequential Bonferroni corrections at the $\alpha = 0.05$ level. We performed a Mantel's test using GENALEX software v. 6.1 (Peakall & Smouse 2006) to test for a correlation between genetic distance and geographic distance.

Clustering analysis

We used the program STRUCTURE (Pritchard *et al.* 2000) to further examine levels of genetic population structure across all sampled localities. STRUCTURE assigns individuals to subpopulations (k) independent of sampling sites and uses a Bayesian clustering method to assign individuals with similar multilocus genotypes to

Table 2. Descriptive statistics for six *Lottia gigantea* microsatellite loci across eight sampled field localities. Given are the number of samples (N), the observed (H_o) and expected heterozygosities (H_e) and the number of alleles (A) found per loci and locality. No significant evidence of heterozygote deficit was found for a multilocus test across localities. However, *Lg6* was found to deviate significantly from HWE using an exact test for each locus (multilocality; see text for details).

	Lg1$(ATC)_n$				*Lg2*$(AAC)_n$				*Lg3*$(AAC)_n$				*Lg4*$(TGA)_n$				*Lg5*$(AAC)_n$				*Lg6*$(GTT)_n$				Het. def.	
Locality (°N)	N	H_e	H_o	A	N	H_e	H_o	A	N	H_e	H_o	A	N	H_e	H_o	A	N	H_e	H_o	A	N	H_e	H_o	A	P (SE)	
Cabrillo N.M. (32.66)	28	0.76	0.82	10	28	0.80	0.75	9	26	0.92	0.96	15	28	0.91	0.79	13	28	0.88	0.86	13	22	0.91	0.91	14	0.16 (0.00)	
La Jolla (32.87)	35	0.81	0.80	11	25	0.68	0.80	8	32	0.91	0.91	17	35	0.93	1.0	15	36	0.92	0.97	14	32	0.92	0.84	15	0.84 (0.00)	
Palos Verdes (33.71)	35	0.74	0.77	10	31	0.68	0.68	8	31	0.91	0.94	17	37	0.90	0.95	14	46	0.89	0.93	14	38	0.92	0.89	15	0.85 (0.00)	
Vandenberg (34.73)	23	0.85	0.83	12	25	0.56	0.56	9	24	0.89	0.83	14	22	0.91	0.95	11	24	0.91	0.83	15	25	0.91	0.84	15	0.22 (0.00)	
Ken Norris (35.52)	19	0.69	0.63	7	19	0.62	0.79	6	18	0.88	0.94	14	18	0.93	0.89	12	19	0.93	0.89	14	19	0.90	0.84	14	0.11 (0.00)	
Pebble Beach (36.56)	28	0.75	0.71	8	25	0.68	0.68	7	27	0.90	0.88	13	27	0.91	0.96	12	28	0.90	0.82	14	24	0.91	0.88	14	0.11 (0.00)	
Bolinas (37.89)	18	0.83	0.78	8	18	0.54	0.55	7	18	0.91	0.94	12	18	0.92	0.89	11	16	0.94	0.94	13	17	0.92	0.82	14	0.13 (0.00)	
Pt. Reyes (38.18)	14	0.83	0.79	7	13	0.50	0.54	4	15	0.86	0.93	11	15	0.90	1.0	9	14	0.91	1.0	12	15	0.89	0.87	12	0.89 (0.00)	
Het. def. P (SE)	0.81 (0.02)				0.62 (0.02)				0.87 (0.02)				0.88 (0.01)				0.29 (0.03)				0.00 (0.00)					
Sum	200			12	184			10	191			22	191			19	211			20	192					

probable subpopulations of origin. We used two default models that are considered to be the most biologically realistic: (i) the admixture model, which assumes that individuals have mixed ancestry (*i.e.* individual i has inherited some proportion of its genome from its ancestors in population k); and (ii) the uncorrelated allele frequency model, which assumes that allele frequencies are independent draws from a distribution specified by the parameter λ. We performed 10 runs for each k (1–10) with a burnin of 30,000 steps followed by 100,000 Markov Chain Monte Carlo (MCMC) iterations to estimate the mean and variance of log likelihoods and posterior probabilities of the number of assumed populations. For each run, we inspected the time series plots to confirm that they reached a stable stationary distribution. We did not provide the program with *a priori* information about the population of origin for each individual. The best estimate of k that maximized the posterior probability of the data was determined by averaging the maximum likelihood scores for each k value. STRUCTURE also provides an estimate of the probability of the mean proportion of membership (q) for each individual within the assigned subpopulation. Individuals with q values = 0.90 or greater are considered to be confidently placed into their subpopulation of origin (Pritchard *et al.* 2000).

Results

We found no evidence of linkage disequilibrium between any of the loci across all populations after sequential Bonferroni corrections (at the $\alpha = 0.05$ level). In addition, we found no evidence of deviation from HWE across sites (multilocus test) as indicated by the non-significant P-values for heterozygote deficit in Table 2. However, we did find evidence of significant deviation from HWE for the *Lg6* locus (multisite test; P = 0.0005) after sequential

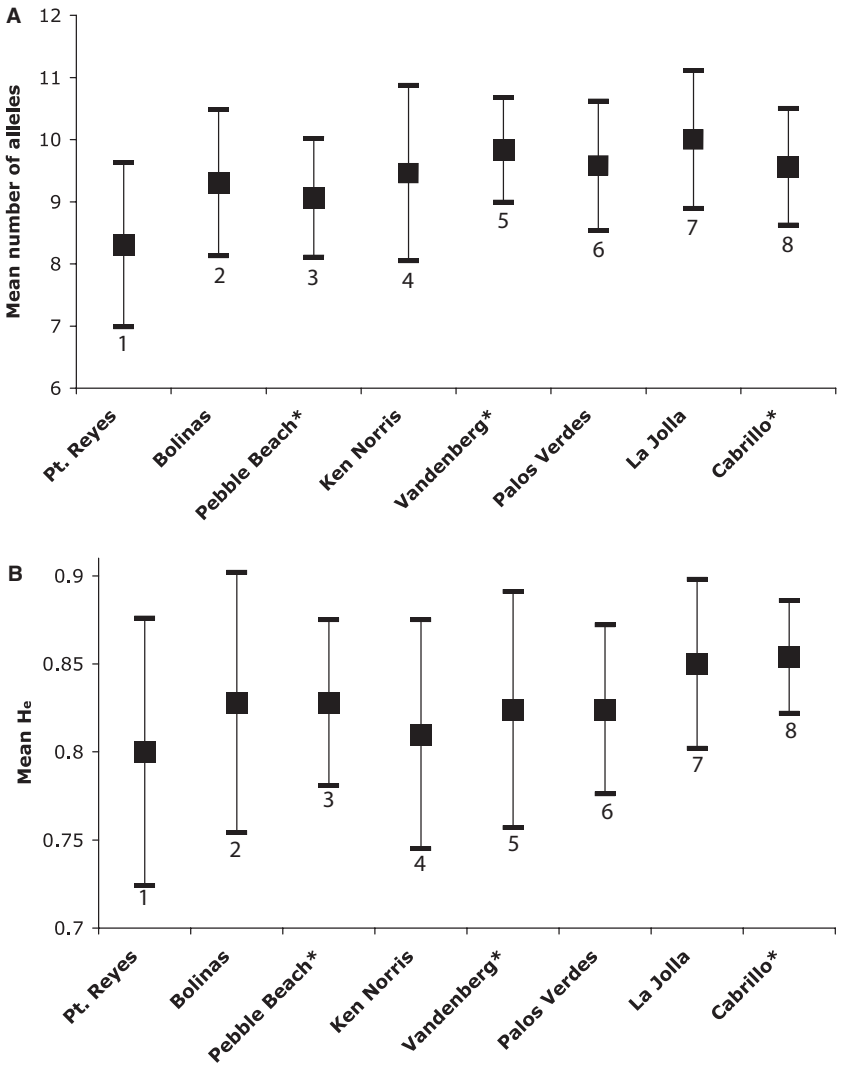

Fig. 2. Mean allelic richness (based on minimum sample size) (A) and mean expected heterozygosity (B) for sampled populations. Error bars represent the standard error. Protected populations are marked with asterisks. Numbers (1–8) refer to the sampled localities in Fig. 1. Pairwise comparisons reveal no significant differences in allelic richness or expected heterozygosity across all populations.

Table 3. Global and pairwise comparisons of genotypic differentiation and F-statistics (F_{st} and ρ_{st}).

	Genotypic differentiation	F-statistics	
Comparison	P-value	F_{st}	ρ_{st}
Global	**0.284**	**0.002**	**−0.003**
BOL & PR	0.175	−0.001	−0.007
PBL & PR	0.455	−0.004	−0.001
PBL & BOL	0.747	−0.004	0.005
KN & PR	0.133	0.006	−0.003
KN & BOL	0.363	0.003	0.001
KN & **PBL**	0.325	−0.005	−0.009
VBG & PR	0.610	−0.004	−0.001
VBG & BOL	0.535	−0.005	−0.010
VBG & **PBL**	0.710	−0.002	−0.013
VBG & KN	0.310	0.000	−0.022
PV & PR	0.147	0.012	0.024
PV & BOL	0.418	0.001	0.032
PV & **PBL**	0.586	−0.004	0.005
PV & KN	0.506	0.000	−0.015
PV & **VBG**	0.910	0.000	−0.004
LJ & PR	0.092	0.008	−0.004
LJ & BOL	0.814	−0.004	−0.012
LJ & **PBL**	0.328	−0.001	−0.009
LJ & KN	0.667	−0.001	−0.008
LJ & **VBG**	0.648	0.001	−0.014
LJ & PV	0.939	0.000	0.013
CNM & PR	0.002	0.022	−0.005
CNM & BOL	0.154	0.012	0.002
CNM & **PBL**	0.215	0.002	−0.002
CNM & KN	0.074	0.009	−0.019
CNM & **VBG**	0.094	0.013	−0.017
CNM & PV	0.233	0.004	−0.005
CNM & LJ	0.350	0.005	0.002

No significant P-values existed after sequential Bonferroni correction at the $\alpha = 0.05$ level. Protected populations are in bold.

Bonferroni correction. This may be due to any number of factors, including null-alleles, non-random mating, inbreeding or selection (Lowe *et al.* 2004). Although including *Lg6* did not significantly alter any of our results, we chose to exclude this locus for the remainder of our analyses given the possibility that it is not a neutral Mendelian marker.

The five remaining tri-nucleotide repeat loci each contained 10–22 alleles (Table 2). The mean number of alleles per locus within a population ranged from 8.6 to 13.0, with the lowest value belonging to the northernmost population at Pt. Reyes. Mean expected heterozygosity (H_e) values ranged from 0.80 to 0.85. Based on the minimum sample size (Table 2), the mean number of alleles per population ranges from 8.3 to 10.0. Pairwise *t*-tests of mean allelic richness (based on minimum sample size) and mean expected heterozygosity performed across populations revealed no significant differences in genetic diversity (Fig. 2).

Analysis of all loci combined revealed no significant global differentiation among populations (P = 0.284). In addition, no significant pairwise divergence between populations was found (exact tests and through F_{st} and ρ_{st}) after sequential Bonferroni corrections (Table 3). Furthermore, the calculated standardized values for F_{st} (F'_{st}) revealed non-significant results (overall $F'_{st} = 0.012$). Similarly, the results from the Mantel test show that there is no significant correlation between geographic distance and genetic distance across populations (P = 0.280).

A lack of significant genetic differentiation between populations is further supported from our clustering analysis using the program STRUCTURE. Regardless of the assumed k, the proportion of membership (q) is equally partitioned into each subpopulation for all individuals. For example, at k = 2, the proportion of membership is equal to 0.50; at k = 1, the proportion of membership is 1.0 (Fig. 3). Individuals with q values greater than 0.90 are considered to be confidently placed into their subpopulation of origin (Pritchard *et al.* 2000); therefore, our results indicate that all genotyped individuals represent part of a single interbreeding population (k = 1).

Discussion

A prerequisite for assessing the genetic effects of harvesting is the establishment of population genetic substructure of the target species (Coltman *et al.* 2003; Allendorf *et al.* 2008). Our analyses reveal no such structure or differences in genetic diversity among local populations of *Lottia gigantea* from throughout the California mainland portion of its geographic range. These results suggest high gene flow between populations and a lack of long-term geographic barriers to dispersal. This is consistent with the pattern seen in other coastal marine invertebrates with planktonic larvae, where low population differentiation and high gene flow between populations is commonly reported (Hellberg 1996; Dawson 2001; Gruenthal *et al.* 2007; Kelly & Eernisse 2007; Lee & Boulding 2007; Addison *et al.* 2008). For harvested species, our results are similar to those found for the California populations of the harvested red abalone, *Haliotis rufescenes* (Gruenthal *et al.* 2007). *Haliotis rufescenes* and *L. gigantea* have similar geographic ranges, a comparable early life history, and a lack of significant genetic subdivision among populations using the same number (5) of microsatellite markers covering roughly the same geographic area.

Given that our analysis indicates extensive contemporary gene flow across most of the geographic range of *L. gigantea*, the exploited populations of this species do not appear to be genetically differentiated from the protected populations. On the other hand, multiple studies have shown that there are clear and significant non-genetically measured

differences (*e.g.* body size, individual growth rates, size at sex change, abundance) between exploited and protected populations of this species (Pombo & Escofet 1996; Lindberg *et al.* 1998; Roy *et al.* 2003; Sagarin *et al.* 2007; Fenberg 2008). Thus these differences are likely to represent a largely plastic (non-genetic) response to size-selective harvesting. From a conservation perspective, this is promising because it indicates that if proper actions are taken to reduce illegal harvesting (which constitutes the vast majority of harvest for this species; California Fish and Game personal communication, Brooke McVeigh), then exploited populations should be able to return to their pre-impact state given sufficient time. This hypothesis has been proposed before (Kido & Murray 2003; Roy *et al.* 2003), but not tested using genetic data.

Alternatively, the absence of contemporary genetic population differentiation may itself be a result of harvesting, compounded by the high-dispersal capabilities of *L. gigantea*. Harvesting can lead to an increase in gene flow to exploited populations from those less affected. This can occur when harvesting initially reduces the density of local populations, allowing for increased immigration from neighboring populations. This may lead to genetic swamping in which genetic differences between populations are reduced, potentially limiting the ability of local populations to adapt to exploitation (Allendorf *et al.* 2008). This hypothesis is difficult to test given the lack of information about the genetic population structure of *L. gigantea* before most of the California populations of this species were affected by harvesting.

Certain aspects of California coastal oceanography combined with the life history of *L. gigantea* may also contribute to high population connectivity. During the winter spawning season for *L. gigantea*, currents along this coast generally flow northwards (Winant *et al.* 2003; Shanks & Eckert 2005; Gruenthal *et al.* 2007), making the region near the northern geographic range limit of this species a possible larval sink. In such a case, population persistence at the northern end of the range could be sustained by larvae from more southerly populations, even if they arrived only sporadically during favorable years. For long-lived species such as *L. gigantea*, unidirectional but sporadic recruitment events can have lasting genetic and demographic effects on a local population (Gruenthal *et al.* 2007; Fenberg 2008). In fact, comparative ecological surveys suggest recruitment is sporadic in northern populations of *L. gigantea*. Northern populations are characterized by low adult abundances and an almost complete lack of juveniles, in stark contrast to Central and Southern Californian populations (Fenberg 2008). Considering that *L. gigantea* individuals can reach ages of 20 years (Fenberg 2008), unidirectional and sporadic recruitment events on the decadal scale may be all that is needed to maintain genetic homogeneity and the existence of these fringe 'populations'. Sampling of specific recruitment events and/or age cohorts can provide better insights into the role of such sporadic recruitment (Flowers *et al.* 2002; Hellberg *et al.* 2002) but this remains a challenge for *L. gigantea* because the larvae of this species are not commonly seen in the field and newly settled individuals can be cryptic, often settling in mussel beds, and are very difficult to distinguish from those of other limpets.

We cannot rule out the possibility that the lack of genetic differentiation among populations seen here may be due to

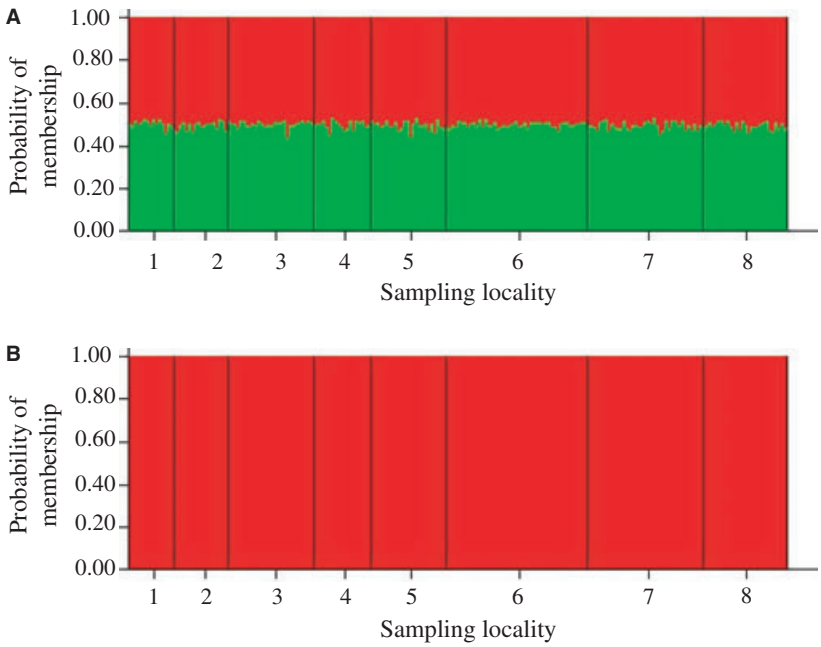

Fig. 3. STRUCTURE bar plots of the proportion of membership (q) for individuals from sampled localities for assumed subpopulations (A) k = 2 and (B) k = 1. The proportion of membership is equally partitioned into each subpopulation (k = 1–10) for all individuals, with a k = 1 having the highest proportion of membership (q = 1.0). The numbered sampled localities (1–8) are arranged by latitude as shown in Fig. 1.

the lack of power in the microsatellite data. Recent analyses of the California red abalone, *Haliotis rufescenes*, also failed to find significant differentiation among populations using five microsatellite loci, but did find some evidence for divergence using 142 AFLP markers, which greatly increased the power of the analyses (Gruenthal *et al.* 2007).

In summary, the lack of significant genetic differences between populations of *L. gigantea* suggests that the response of this species to size-selective harvesting pressure largely represents phenotypic plasticity. Untangling genetic and plastic effects, however, is notoriously difficult (Allendorf *et al.* 2008; Fenberg & Roy 2008), especially for broadcast spawning marine invertebrates where conventional mitochondrial and/or nuclear markers may not always have enough power to resolve population subdivisions (Gruenthal *et al.* 2007; Burton 2009). In the future, higher resolution studies using many more loci and/or genome-wide analyses and candidate genes (Hemmer-Hansen *et al.* 2007; Allendorf *et al.* 2008; Burton 2009) may provide a more refined insight into this issue.

Finally, the lack of genetic structure seen here should not preclude the adoption of evolutionary sustainable management practices for *L. gigantea*. Effective management of this species needs to take into account not only these genetic data, but also the history, ecology, biogeography, and life history parameters (Pombo & Escofet 1996; Lindberg *et al.* 1998; Kido & Murray 2003; Roy *et al.* 2003; Sagarin *et al.* 2007; Fenberg 2008). Proper enforcement to curb illegal size-selective harvesting is the first and most important step in returning exploited populations to their pre-impact state (Roy *et al.* 2003). Only then can we know for sure whether size-selective harvest has had a long-term impact on the biology of *Lottia gigantea*.

Acknowledgements

This work was supported by an EPA STAR Fellowship (Environmental Protection Agency) to P.B.F. and a NOAA California SeaGrant to K.R. We would also like to thank the National Park Service and the University of California Reserve System for research permission at Cabrillo National Monument, the Scripps Coastal Reserve, and the Kenneth S. Norris Rancho Marino Reserve. We are grateful for the help of two anonymous reviewers on a previous version of this paper.

References

Addison J.A., Ort B.S., Mesa K.A., Pogson G.H. (2008) Range-wide genetic homogeneity in the California sea mussel (*Mytilus californianus*): a comparison of allozymes, nuclear DNA markers, and mitochondrial DNA sequences. *Molecular Ecology*, **17**, 4222–4232.

Allendorf F.W., England P.R., Luikart G., Ritchie P.A., Ryman N. (2008) Genetic effects of harvest on wild animal populations. *Trends in Ecology and Evolution*, **23**, 327–337.

Banks S.C., Piggott M.P., Williamson J.E., Bove U., Holbrook N.J., Beheregaray L.B. (2007) Oceanic variability and coastal topography shape genetic structure in a long-dispersing sea urchin. *Ecology*, **88**, 3055–3064.

Burton R.S. (1998) Intraspecific phylogeography across the Point Conception biogeographic boundary. *Evolution*, **52**, 734–745.

Burton R.S. (2009) Molecular markers, natural history and conservation of marine animals. *BioScience*, **59**, 831–841.

Chevolot M., Ellis J.R., Rijnsdorp A.D., Stam W.T., Olsen J.L. (2008) Temporal changes in allele frequencies but stable genetic diversity over the past 40 years in the Irish Sea population of thornback ray, *Raja clavata*. *Heredity*, **101**, 120–126.

Coltman D.W., O'Donoghue P., Jorgenson J.T., Hogg J.T., Strobeck C., Festa-Bianchet M. (2003) Undesirable evolutionary consequences of trophy hunting. *Nature*, **426**, 655–658.

Daly G. (1975) Growth and reproduction in the marine limpet *Lottia gigantea* (Gray) (Acmaeidae). MS Thesis, San Diego State University.

Dawson M.N. (2001) Phylogeography in coastal marine animals: a solution from California? *Journal of Biogeography*, **28**, 723–736.

Fenberg P.B. (2008) The effects of size-selective harvesting on the population biology and ecology of a sex-changing limpet species, *Lottia gigantea*. PhD Dissertation, University of California, San Diego.

Fenberg P.B., Roy K. (2008) Ecological and evolutionary consequences of size-selective harvesting: how much do we know? *Molecular Ecology*, **17**, 209–220.

Flowers J.M., Schroeter S.C., Burton R.S. (2002) The recruitment sweepstakes has many winners: genetic evidence from the sea urchin *Strongylocentrotus purpuratus*. *Evolution*, **56**, 1445–1453.

Glickman T.S. (2000) *Glossary of Meteorology*. American Meteorological Society, Cambridge: 855 pp.

Goudet J. (1995) FSTAT (version 1.2): a computer program to calculate F-statistics. *Journal of Heredity*, **86**, 485–486.

Grift R.E., Rijnsdorp A.D., Barot S., Heino M., Dieckmann U. (2003) Fisheries-induced trends in reaction norms for maturation in North Sea plaice. *Marine Ecology Progress Series*, **257**, 247–257.

Gruenthal K.M., Burton R.S. (2008) Genetic structure of natural populations of the California black abalone (*Haliotis cracherodii* Leach, 1814), a candidate for endangered species status. *Journal of Experimental Marine Biology and Ecology*, **355**, 47–58.

Gruenthal K.M., Acheson L.K., Burton R.S. (2007) Genetic structure of natural populations of California red abalone (*Haliotis rufescens*) using multiple genetic markers. *Marine Biology*, **152**, 1237–1248.

Hauser L., Adcock G.J., Smith P.J., Ramirez J.H.B., Carvalho G.R. (2002) Loss of microsatellite diversity and low effective

population size in an overexploited population of New Zealand snapper (*Pagrus auratus*). *Proceedings of the National Academy of Sciences of the United States of America*, **99**, 11742–11747.

Heath D.D., Busch C., Kelly J., Atagi D.Y. (2002) Temporal change in genetic structure and effective population size in steelhead trout (*Oncorhynchus mykiss*). *Molecular Ecology*, **11**, 197–214.

Hedrick P.W. (2005) A standardized genetic differentiation measure. *Evolution*, **59**, 1633–1638.

Hellberg M.E. (1996) Dependence of gene flow on geographic distance in two solitary corals with different larval dispersal capabilities. *Evolution*, **50**, 1167–1175.

Hellberg M.E., Burton R.S., Neigel J.E., Palumbi S.R. (2002) Genetic assessment of connectivity among marine populations. *Bulletin of Marine Science*, **70**, 273–290.

Hemmer-Hansen J., Nielsen E.E., Frydenberg J., Loeschcke V. (2007) Adaptive divergence in a high gene flow environment: Hsc70 variation in the European flounder (*Platichthys flesus* L.). *Heredity*, **99**, 592–600.

Hutchinson W.F., van Oosterhout C., Rogers S.I., Carvalho G.R. (2003) Temporal analysis of archived samples indicates marked genetic changes in declining North Sea cod (*Gadus morhua*). *Proceedings of the Royal Society Biological Sciences Series B*, **270**, 2125–2132.

Jerardino A., Castilla J.C., Ramirez J.M., Hermosilla N. (1992) Early coastal subsistence patterns in central Chile: a systematic study of the marine invertebrate fauna from the site of Curaumilla-1. *Latin American Antiquity*, **3**, 43–62.

Kay M.C., Emlet R.B. (2002) Laboratory spawning, larval development, and metamorphosis of the limpets *Lottia digitalis* and *Lottia asmi* (Patellogastropoda, Lottiidae). *Invertebrate Biology*, **121**, 11–24.

Kelly R.P., Eernisse D.J. (2007) Southern hospitality: a latitudinal gradient in gene flow in the marine environment. *Evolution*, **61**, 700–707.

Kido J.S., Murray S.N. (2003) Variation in owl limpet *Lottia gigantea* population structures, growth rates, and gonadal production on southern California rocky shores. *Marine Ecology Progress Series*, **257**, 111–124.

Kuparinen A., Merila J. (2007) Detecting and managing fisheries-induced evolution. *Trends in Ecology and Evolution*, **22**, 652–659.

Lee H.J., Boulding E.G. (2007) Mitochondrial DNA variation in space and time in the northeastern Pacific gastropod, *Littorina keenae*. *Molecular Ecology*, **16**, 3084–3103.

Lindberg D.R., Estes J.A., Warheit K.I. (1998) Human influences on trophic cascades along rocky shores. *Ecological Applications*, **8**, 880–890.

Lowe A., Harris S., Ashton P. (2004) *Ecological Genetics: Design, Analysis, and Application*. Blackwell Publishing, Maldon: 344 pp.

Mannino A.M., Thomas K.D. (2002) Depletion of a resource? The impact of prehistoric human foraging on intertidal mollusc communities and its significance for human settlement, mobility and dispersal. *World Archaeology*, **33**, 452–474.

Michalakis Y., Excoffier L. (1996) A generic estimation of population subdivision using distances between alleles with special reference for microsatellite loci. *Genetics*, **142**, 1061–1064.

Miller K.J., Maynard B.T., Mundy C.N. (2009) Genetic diversity and gene flow in collapsed and healthy abalone fisheries. *Molecular Ecology*, **18**, 200–211.

Murray S.N., Denis T.G., Kido J.S., Smith J.R. (1999) Human visitation and the frequency and potential effects of collecting on rocky intertidal populations in southern California marine reserves. *California Cooperative Oceanic Fisheries Investigations Reports*, **40**, 100–106.

Nielsen E.E., Hansen M.M., Loeschcke V. (1999) Genetic variation in time and space: microsatellite analysis of extinct and extant populations of Atlantic salmon. *Evolution*, **53**, 261–268.

Nussle S., Bornand C.N., Wedekind C. (2009) Fishery-induced selection on an Alpine whitefish: quantifying genetic and environmental effects on individual growth rate. *Evolutionary Applications*, **2**, 200–208.

Olsen E.M., Heino M., Lilly G.R., Morgan M.J., Brattey J., Ernande B., Dieckmann U. (2004) Maturation trends indicative of rapid evolution preceded the collapse of northern cod. *Nature*, **428**, 932–935.

Peakall R., Smouse P.E. (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes*, **6**, 288–295.

Perez-Ruzafa A., Gonzalez-Wanguemert M., Lenfant P., Marcos C., Garcia-Charton J.A. (2006) Effects of fishing protection on the genetic structure of fish populations. *Biological Conservation*, **129**, 244–255.

Pombo O.A., Escofet A. (1996) Effect of exploitation on the limpet *Lottia gigantea*: a field study in Baja California (Mexico) and California (U.S.A.). *Pacific Science*, **50**, 393–403.

Pritchard J.K., Stephens M., Donnelly P. (2000) Inference of population structure using multilocus genotype data. *Genetics*, **155**, 945–959.

Raymond M., Rousset F. (1995) GENEPOP (version-1.2) – Population-Genetics Software for exact tests and ecumenicism. *Journal of Heredity*, **86**, 248–249.

Raymond M., Rousset F. (2003) GENEPOP (version 3.4): population genetics software for exact tests and ecumenicism. Updated from Raymond and Rousset (1995), *Journal of Heredity*, **86**, 248–249.

Rousset F. (1996) Equilibrium values of measures of population subdivision for stepwise mutation processes. *Genetics*, **142**, 1357–1362.

Roy K., Collins A.G., Becker B.J., Begovic E., Engle J.M. (2003) Anthropogenic impacts and historical decline in body size of rocky intertidal gastropods in southern California. *Ecology Letters*, **6**, 205–211.

Rozen S., Skaletsky H.J. (2000) PRIMER3 on the WWW for general users and for biologist programmers. In: Krawetc S.,

Misener S. (Eds), *Bioinformatics Methods and Protocols: Methods in Molecular Biology*. Humana Press, Totowa: pp. 365–386.

Ruzzante D.E., Taggart C.T., Doyle R.W., Cook D. (2001) Stability in the historical pattern of genetic structure of Newfoundland cod (*Gadus morhua*) despite the catastrophic decline in population size from 1964 to 1994. *Conservation Genetics*, **2**, 257–269.

Sagarin R.D., Ambrose R.F., Becker B.J., Engle J.M., Kido J., Lee S.F., Miner C.M., Murray S.N., Raimondi P.T., Richards D., Roe C. (2007) Ecological impacts on the limpet *Lottia gigantea* populations: human pressure over a broad scale on island and mainland intertidal zones. *Marine Biology*, **150**, 399–413.

Shanks A.L., Eckert G.L. (2005) Population persistence of California Current fishes and benthic crustaceans: a marine drift paradox. *Ecological Monographs*, **75**, 505–524.

Siegfried W.R., Hockey P.A.R., Branch G.M. (1994) The exploitation of intertidal and subtidal biotic resources of rocky shores in Chile and South Africa. In: Siegfried W.R. (Ed.), *Rocky Shores. Exploitation in Chile and South Africa*. Springer-Verlag, Berlin: pp. 1–15.

Smith P.J., Francis R., McVeagh M. (1991) Loss of genetic diversity due to fishing pressure. *Fisheries Research*, **10**, 309–316.

Trexler J.C., Travis J. (2000) Can marine protected areas restore and conserve stock attributes of reef fishes? *Bulletin of Marine Science*, **66**, 853–873.

Wares J.P., Gaines S.D., Cunningham C.W. (2001) A comparative study of asymmetric migration events across a marine biogeographic boundary. *Evolution*, **55**, 295–306.

Weir B.S., Cockerham C.C. (1984) Estimating F-statistics for the analysis of population-structure. *Evolution*, **38**, 1358–1370.

Winant C.D., Dever E.P., Hendershott M.C. (2003) Characteristic patterns of shelf circulation at the boundary between central and southern California. *Journal of Geophysical Research*, **108**, 1–13.