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RESEARCH ARTICLE

Incorporating historical and ecological genetic data for leopard grouper (*Mycteroperca rosacea*) into marine reserve design in the Gulf of California

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Abstract Effective design of marine reserves for use in fisheries management and conservation requires a clear understanding of patterns of larval transport and sink-source dynamics between populations, as well as a clear understanding of population demography. Mitochondrial and nuclear markers were analyzed to investigate potential mechanisms impacting connectivity among and the demographic history of subpopulations of a commercially important species in the Gulf of California, the leopard grouper (*Mycteroperca rosacea*). Demographic history and connectivity analyses included a coalescent analysis, estimating neutrality indices, estimating global and pairwise F'_{ST}, Φ_{ST}, or G''_{ST}, and a priori methodologies to identify genetically distinct units and barriers to dispersal. Average, long-term connectivity between geographic regions in the Gulf was also estimated. Divergence of mitochondrial lineages of leopard grouper dated to the late Pleistocene, with deep-water islands serving as demographically stable populations that may have acted as sources for new populations during periods of climate variability. Additionally, we observed genetically distinct units of leopard grouper in the Gulf, particularly between peninsular and mainland sites, as well as asymmetrical migration between the northern and central Gulf. Observed patterns of genetic differentiation are likely attributed to complex asymmetrical oceanographic currents and local larval retention. Based on our genetic findings and current fishing pressure in certain regions, we recommend implementing small, upstream no-take zones in the areas east of Isla Ángel de la Guarda, around Isla San Lorenzo and Isla San Esteban, and north of Isla Tiburón, that would enhance connectivity among subpopulations, preserve sites with high genetic diversity, and benefit fisheries downstream of these sites.

Keywords Leopard grouper · *Mycteroperca rosacea* · Gulf of California · Connectivity · Conservation genetics · Marine reserves · Fisheries management

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Introduction

Marine reserves have become an increasingly important tool in fisheries management that, if designed properly, can provide major benefits such as increased biomass for commercially important species (Roberts 1995; Halpern and Warner 2002) and enhancement of adjacent fisheries (Alcala and Russ 1990; Roberts et al. 2001). Effective design of marine reserves for use in fisheries management and conservation requires a clear understanding of patterns of larval transport between populations (Sale et al. 2005; Levin 2006; Gaines et al. 2010). While the small size of larvae make them challenging to track in the marine environment, genetic markers can be used as a proxy to assess larval dispersal patterns, thereby revealing patterns of genetic connectivity (Avise 2004). Phylogeographic and population genetics analyses of such markers then allow us to infer the extent of genetic connectivity among populations (e.g. Pearse and Crandall 2004; Hedgecock et al. 2007), or the degree to which gene flow affects evolutionary processes within populations (Lowe and Allendorf 2010), as well as historical and contemporary mechanisms driving divergence when connectivity is limited (Cowen and Sponaugle 2009). The extent of connectivity among established reserves, and the extent of genetic differentiation among organisms within them, may be influenced by a number of mechanisms including vicariance events (Bernardi et al. 2003; Riginos 2005), environmental differences (Riginos and Nachman 2001), oceanography (Soria et al. 2012; Beldade et al. 2014), and limited dispersal ability (Hurtado et al. 2010).

The Gulf of California is a biodiverse marine eco-region that could greatly benefit from increased conservation and fisheries management efforts (Ezcurra 2002). The Gulf of California is viewed as a conservation priority internationally due to its high levels of species endemism (Roberts et al. 2002), and became a UNESCO World Heritage Site in 2005 due to its striking landscapes, high levels of biodiversity and marine productivity, as well as its unique oceanographic processes. High levels of species diversity observed in this unique eco-region are in part threatened by a lack of fishing regulations in place to mitigate increased fishing pressure from artisanal fisheries (Carvajal et al. 2004; Lluch-Cota et al. 2007; Moreno-Baez et al. 2012). As a result, several researchers, government officials, and non-governmental organizations (NGOs) have proposed establishing networks of marine reserves in the Gulf of California in order to conserve biological diversity in the region, and ten protected areas have already been established (Sala et al. 2002; Rife et al. 2013), as means of averting widespread species declines.

In this study, we used mitochondrial DNA (mtDNA) and microsatellites to investigate potential processes impacting the demographic history and patterns of connectivity among subpopulations of an ecologically important top predator, the leopard grouper (*Mycteroperca rosacea*), in the rocky reefs of the Gulf of California. Leopard grouper is listed as Vulnerable by the International Union for the Conservation of Nature (IUCN) as it is currently the mostly intensely targeted grouper species in subsistence, artisanal, and recreational fishing activities throughout its geographic range. The species is particularly susceptible to overfishing during the season when it aggregates to spawn (Erisman et al. 2010). Despite leopard grouper being the most economically and commercially important grouper in the Gulf of California, there is no species-specific management in place to regulate fishing activities targeting it in the Gulf of California. Results of this study will be used to assess the effectiveness of current reserves in maintaining connectivity among populations of leopard grouper in the Gulf of California, with an effective network of marine reserves providing a means of regulating harvest of leopard grouper, maintaining small scale fisheries and genetic diversity in overharvested populations, and potentially benefiting other species with similar population dynamics and population structure to leopard grouper.

Materials and methods

Leopard grouper are found in rocky reefs distributed from Bahía Magdalena, through the Gulf of California and as far south as Jalisco, Mexico (Thomson et al. 2000). They are gonochoristic and aggregate to spawn in shallow rocky reefs between March and June within groups of 6 to 40 (Erisman et al. 2007). Larvae remain in the water column for approximately 24 days before settling (Aburto-Oropeza et al. 2007).

Sample collection and DNA extraction

We sampled a total of 551 leopard grouper pectoral fins from 21 localities in the Gulf of California (Table 1; Fig. 1) between 2006 and 2012. Sampling occurred at sites throughout the northern (north of Bahía San Francisquito past the southern tip of Isla Tiburón) and central Gulf (Bahía San Francisquito to La Paz and Bahía Kino to Guaymas), as described by Walker (1960) in Thomson et al. (2000). Samples in our study were acquired from localities that fell within 5 of the 10 existing marine reserves in the Gulf of California (described in Rife et al. 2013). The remoteness of some sampling localities, particularly those northern Gulf sites in the Midriff Islands (an archipelago in the northern Gulf comprised of approximately 45 islands and islets [sites 8 −15]), made uniform sampling across all sites challenging, and in some cases resulted in smaller sample sizes. All

Table 1 Molecular diversity indices for mitochondrial DNA for leopard grouper

Sampling site	*n*	nH	h*	π	F_S	D
1. San Felipe^{++}	13	10	0.924	0.0015 ± 0.0010	**−6.695**	**−1.755**
2. Puertecitos	8	7	0.931	0.0019 ± 0.0013	**−3.393**	−1.283
3. San Luis Gonzaga	16	12	0.920	0.0016 ± 0.0010	**−8.513**	−1.707
4. Bahía de los Angeles^{++}	52	23	0.885	0.0016 ± 0.0010	**−17.754**	**−1.985**
5. Puerto Peñasco	25	13	0.858	0.0013 ± 0.0009	**−7.976**	**−1.863**
6. Puerto Lobos	11	7	0.902	0.0018 ± 0.0012	−1.976	−0.061
7. Puerto Libertad	55	21	0.887	0.0016 ± 0.0010	**−13.175**	−1.384
8. Isla Ángel de la Guarda^{++}	25	9	0.872	0.0015 ± 0.0010	−2.201	−0.052
9. Isla San Lorenzo^{++}	11	4	0.844	0.0011 ± 0.0008	−1.673	−0.077
10. Isla San Esteban	4	4	0.943	0.0013 ± 0.0011	**−1.872**	−0.780
11. Isla Tiburón (North)	18	12	0.911	0.0017 ± 0.0011	**−6.882**	−0.960
12. Isla Tiburón (South)	13	8	0.918	0.0023 ± 0.0014	−1.773	−0.966
13. Isla Datil	7	6	0.925	0.0013 ± 0.0010	**−3.409**	−1.129
14. Isla Patos	8	8	0.948	0.0026 ± 0.0017	**−4.670**	−1.096
15. Isla San Pedro Mártir^{++}	17	9	0.881	0.0014 ± 0.0010	**−3.648**	−1.498
16. La Jerga	23	17	0.920	0.0019 ± 0.0012	**−13.177**	**−1.896**
17. Isla San Marcos	51	20	0.845	0.0014 ± 0.0009	**−14.446**	**−2.060**
18. Isla Carmen^{++}	53	23	0.902	0.0017 ± 0.0011	**−15.850**	**−1.617**
19. Isla Cerralvo	51	23	0.901	0.0018 ± 0.0011	**−16.194**	**−1.834**
20. Bahía Kino	28	18	0.918	0.0016 ± 0.0010	**−14.726**	**−1.671**
21. Isla San Pedro Nolasco	62	32	0.918	0.0018 ± 0.0011	**−26.534**	**−1.903**

Sample location, number of specimens (*n*), number of haplotypes (nH), corrected haplotype diversity (h*), nucleotide diversity (π) and the neutrality statistic Fu's F_S and Tajima's D. Bolded values denote statistical significance of $p < 0.05$ for F_S and D values. $^{++}$ denotes sampling localities within a marine reserve

samples from the Midriff Islands were acquired on site, with the assistance of fishermen in small outboard vessels (pangas), due to the fine spatial scale over which sampling occurred. The remaining tissue samples were collected either at fish markets or directly from fishermen at harbors, and the approximate locality where each fish was caught was recorded. Samples were stored in 95 % ethanol and kept at −20 °C in the laboratory. DNA was extracted by incubating 20 mg of tissue in a proteinase K digestion in lysis buffer (10 mM Tris, 400 mM NaCl, 2 mM EDTA, 1 % SDS) overnight at 55 °C. Tissue digestion was followed by purification using standard phenol/chloroform and alcohol precipitation protocols (Sambrook et al. 1989).

Genotyping and data analysis for mitochondrial markers

We genotyped samples for two mitochondrial markers: ATPase and cytochrome *b*. Each 100 µl reaction contained 10–100 ng of DNA, 10 mM Tris HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 2.5 units of Taq DNA polymerase (Perkin-Elmer), 50 mM of each dNTP, and 0.3 mM of each primer. We amplified a 726-bp fragment of ATPase using species-specific primers (MYCROS Forward: 5′-TTCTCCCACTACCCTGATTC and MYCROS Reverse: 5′-TACGTAGGCTTGGATCATTG). We amplified a 787-bp fragment of cytochrome *b* using primers Gludgl and CB3H (Palumbi et al. 1991). Polymerase chain reaction (PCR) parameters used to amplify ATPase and cytochrome *b* were the same as those used in Munguía-Vega et al. (2014). PCR products were sequenced on an ABI 3730xl DNA analyzer. ATPase and cytochrome *b* sequences were proofread and aligned using Geneious version 5.6, and subsequently analyzed as concatenated sequences as variation observed in both markers was best explained by the same DNA substitution model in JModeltest 0.1.1 (Posada 2008).

We calculated nucleotide diversity (π), and corrected haplotype diversity (h*) using a rarefaction approach implemented in CONTRIB (Petit et al. 1998) to account for differences in sample size between localities (minimum $n = 4$). Phylogenetic relationships among sequences were conveyed by generating a haplotype network in R using HaploNet in the APE package (Paradis et al. 2004). Haplotype frequencies generated in Arlequin version 3.5 (Excoffier et al. 2005) were used to generate pie diagrams for each haplotype in the network.

Genotyping and data analysis for microsatellite markers

All samples were genotyped for 12 species-specific microsatellites (Mros01–Mros12) following protocols described in Jackson et al. (2013). PCR products were sized on an ABI 3730xl DNA analyzer using LIZ-500 size standard (Applied Biosystems). Microsatellites were scored using GeneMapper version 3.7 (Applied Biosystems), and we tested for null alleles, large allele dropout and scoring

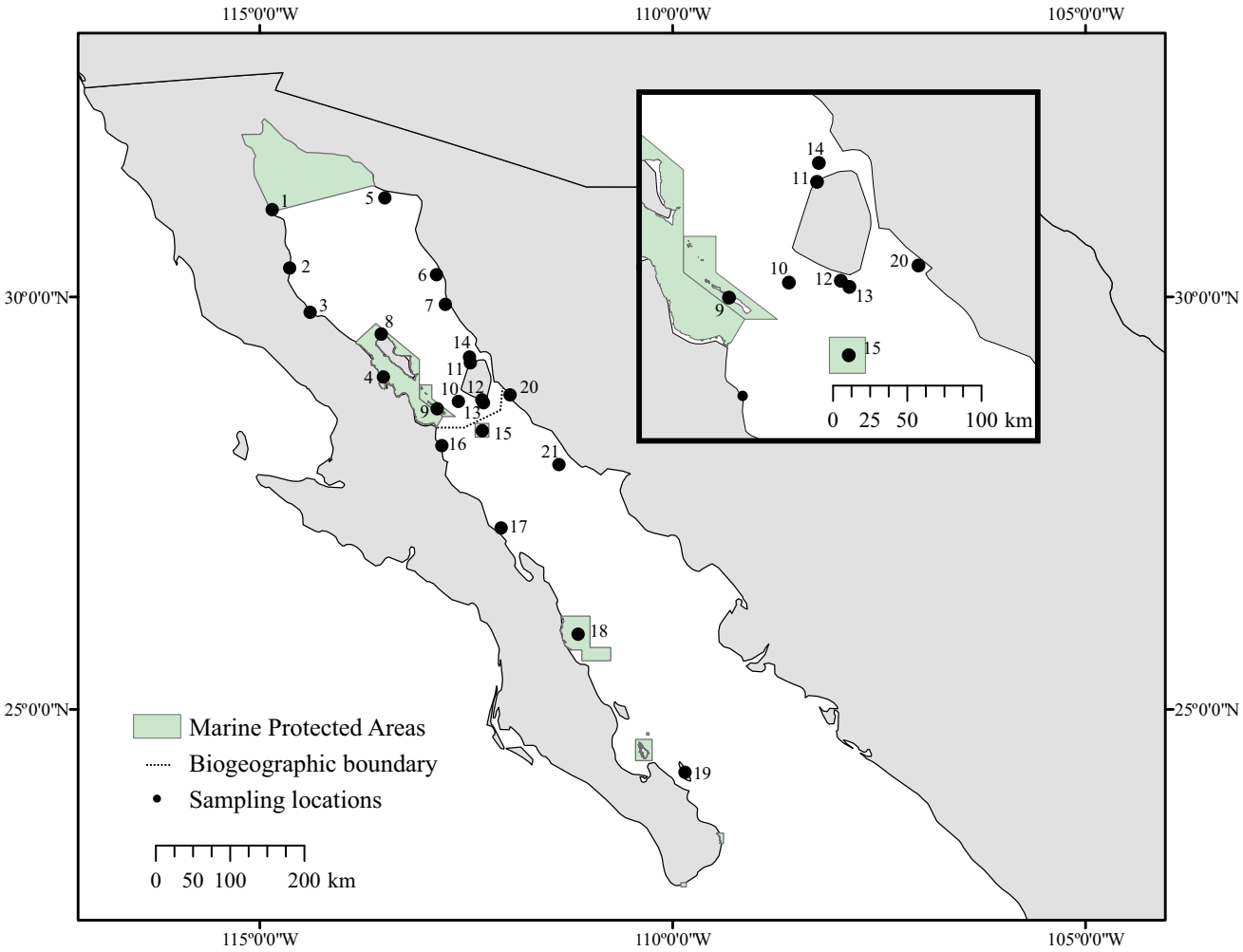

Fig. 1 Leopard grouper sampling localities in the Gulf of California. Sampling localities include: San Felipe (*1*), Puertecitos (*2*), San Luis Gonzaga (*3*), Bahía de los Angeles (*4*), Puerto Peñasco (*5*), Puerto Lobos (*6*), Puerto Libertad (*7*), Isla Ángel de la Guarda (*8*), Isla San Lorenzo (*9*), Isla San Esteban (*10*), Isla Tiburón (North) (*11*), Isla Tiburón (South) (*12*), Isla Datil (*13*), Isla Patos (*14*), Isla San Pedro Martír (*15*), La Jerga (*16*), Isla San Marcos (*17*), Isla Carmen (*18*), Isla Cerralvo (*19*), Bahía Kino (*20*) and Isla San Pedro Nolasco (*21*). Marine protected areas are *shaded* in *green*. The biogeographic boundary between the northern and central Gulf described in Thomson et al. (2000) is marked with a *dashed line*. (Color figure online)

errors using Micro-Checker version 2.2 (Van Oosterhout et al. 2004). We calculated the number of alleles per locus (nA), effective number of alleles per locus (Ae), expected heterozygosity (H_e), observed heterozygosity (H_o), linkage disequilibrium (LD) between pairs of loci, and performed exact tests to detect deviations from Hardy–Weinberg equilibrium (HWE) using Arlequin.

Divergence estimates and demographic patterns

We used JModeltest 0.1.1 to determine the most appropriate DNA substitution model using Akaike Information Criterion (AIC). AIC indicated SYM + I as the best fit model for cytochrome *b* sequence evolution. We calibrated a Bayesian skyline plot using a lognormal relaxed clock in BEAST version 1.7.5 (Drummond et al. 2012) to estimate divergence times. We excluded ATPase from this analysis, as mutation rates were not available. Coalescent approaches allowed us to explore demographic patterns as well as time to most recent common ancestor (tmrca of mtDNA) for all leopard grouper mitochondrial lineages. We used literature-derived mutation rates for cytochrome *b* (Craig et al. 2009), allowing them to vary between 0.5 and 2 % per million years. We performed three replicate runs, sampling every 1,000 steps along a MCMC chain of length 50,000,000 after a burn-in of 15 %.

We estimated Fu's F_S and Tajima's D in order to detect signatures of recent population expansions. When F_S is negative, the number of segregating sites is greater than the

pairwise nucleotide diversity, suggesting an excess of neutral mutations common after a population expansion or selective sweep (Fu 1997). When D is negative, there is often a single allele found in high frequency along with a large number of rare alleles. Such an allele frequency distribution is common in populations that are expanding (Hartl and Clark 2007).

Population structure

We first estimated global F'_{ST}, Φ_{ST}, or G''_{ST}. F'_{ST} is a standardized index which measures genetic differentiation and reaches its maximum when no alleles are shared between populations (Meirmans and Hedrick 2011). Φ_{ST} is an analogue of F_{ST} that incorporates genetic distances between sequences (Excoffier et al. 1992) and G''_{ST} is an unbiased estimator of population structure (Meirmans and Hedrick 2011). Indices were estimated using Arlequin version 3.5 and Genodive version 2.0b23 (Meirmans and Van Tienderen 2004). The statistical power of indices was assessed using the software POWSIM version 4.1 (Ryman and Palm 2006) to address issues associated with detecting small genetic differences in allele frequencies with variable sample sizes across sampling localities. Statistical significance of pairwise F'_{ST}, Φ_{ST}, or G''_{ST} values was assessed after Bonferroni correction (family-wise error rate = 0.050, corrected p value = 0.002). We also performed partial Mantel tests (including all sampling localities) using the vegan package in R to assess the correlation between geographic distance and genetic distance, while controlling for the effect of hierarchical population structure.

We used two a priori methodologies to identify genetically differentiated subpopulations. We first used a computational geometry approach implemented in Barrier version 2.2 (Manni et al. 2004) using the mtDNA and microsatellite data sets. Delaunay triangulation and Voronoi tessellation are used to visualize the geographic locations of barriers to larval dispersal. Ranking and strength of observed barriers were determined based on methods described in Manni et al. (2004). Next we used a Bayesian clustering algorithm to assign individuals to *K* groups (1–21) using the software STRUCTURE version 2.3.4 (Pritchard et al. 2000) using the microsatellite data set. Convergence of parameters (α, F, and likelihood) in preliminary runs was used to determine a suitable burn-in period (500,000) and MCMC chain length for each run (1,000,000). We performed 10 replicate runs for each value of K using an admixture model, and estimated the most likely *K* from STRUCTURE runs using the ΔK and ΔF_{ST} methods implemented in CORRSIEVE (Campana et al. 2011).

Migration patterns

We estimated patterns of average, long-term connectivity between the northern and central Gulf (as defined by Walker's biogeographic regions) for the mtDNA and microsatellite datasets using the Bayesian approach implemented in Migrate-n version 3.5.1 (Beerli and Felsenstein 1999). Run conditions were as follows: Brownian motion mutation model, 5,000,000 recorded steps, a burn-in of 1,000,000 steps, a static heating scheme using 20 temperatures, a tree swapping interval of 1, and an upper prior boundary for migration set to 7,500. We assessed convergence across three replicate runs by recording both effective sample size (ESS) and autocorrelation values.

Results

Genetic data: mitochondrial markers

We identified 127 unique haplotypes, which formed a star-like network (Table 1, Fig. S1). The average distance between haplotypes was 1–2 bp, and the two most abundant haplotypes were observed in 26.9 and 9.1 % of the mtDNA sequences, respectively. The number of haplotypes (nH) and corrected haplotype diversity (h*) are reported in Table 1. Corrected haplotype diversity values were largest in Isla Patos and Isla San Esteban, and lowest in Isla San Marcos and Puerto Peñasco.

Genetic data: microsatellite markers

We excluded four loci (Mros01, Mros06, Mros08, Mros09) from our analysis due to evidence of null alleles and significant linkage disequilibrium. Estimates of number of alleles, observed heterozygosity, expected heterozygosity, and deviations from Hardy–Weinberg equilibrium for the remaining 8 loci are reported in Table S1. The total number of alleles per locus per site ranged from 6 to 40. Significant departures from HWE were observed in 4 out of 168 exact tests ($p < 0.0003$) after Bonferroni correction (Table S2).

Divergence estimates and demographic patterns

All leopard grouper mtDNA sequences coalesced to a most recent common ancestor that dated to the end of the Pleistocene epoch (approximately 120,000–11,800 years ago). Two major clades diverged approximately 113,000 years ago and the majority of mitochondrial lineages within each clade diverged less than 20,000 years ago (Fig. S2). There was no evidence of geographic clustering of samples within clades based on the coalescent tree.

Negative values of Fu's F_S and Tajima's D suggest that most leopard grouper subpopulations are undergoing an expansion. Fu's F_S was significantly negative in 17 of 21 sampling localities, with the exception of Puerto Lobos and sites in the Midriff Islands including Isla Ángel de la Guarda, Isla San Lorenzo and Isla Tiburón (South) (Table 1; Fig. 2). Tajima's D was significantly negative in 9 of 21 sampling localities. All D values in the central Gulf were statistically significant, a few in the northern Gulf, but none in the Midriff Islands (Table 1).

Population structure

We detected statistically significant genetic differentiation using mtDNA (global $F'_{ST} = 0.011$, $p < 0.001$; global $\Phi_{ST} = 0.044$, $p < 0.001$) and microsatellites (global $F'_{ST} = 0.010, p = 0.028$; global $G''_{ST} = 0.072, p < 0.001$). Hypervariability of the microsatellites may explain the order of magnitude difference in the degree of genetic subdivision uncovered between F_{-ST-} versus G''_{ST} (Table S1). Power analyses also indicated our ability to detect observed levels of genetic differentiation with a probability of 99 % for our mtDNA dataset and a probability of 95 % for our microsatellite dataset. Thus, despite small sample sizes for some localities and a relatively low number of microsatellite markers, there was sufficient statistical power in the data set as a whole in order to detect genetic differentiation at the magnitudes that we observed in the Gulf of California for leopard grouper.

After Bonferroni correction of the mtDNA data, there were a larger number of statistically significant pairwise F_{ST} (126 of 210) versus Φ_{ST} pairwise comparisons (6 of 210) (Table S3). While pairwise F_{ST} estimates did not show clear geographic trends, Φ_{ST} values showed large differences between Puerto Libertad in comparison to Bahía de los Angeles and Isla Tiburón (North), and for Isla San Pedro Nolasco in comparison to Bahía de los Angeles, Isla Tiburón (North) and Isla San Pedro Martír. For the microsatellite data set, only 6 out of 210 pairwise F_{ST} were statistically significant after Bonferroni correction (Table S4). All significant F_{ST} values included Isla Tiburón (North) against peninsular sites both in the northern and central Gulf and none showed geographic trends. The largest pairwise G''_{ST} values were observed in pairwise

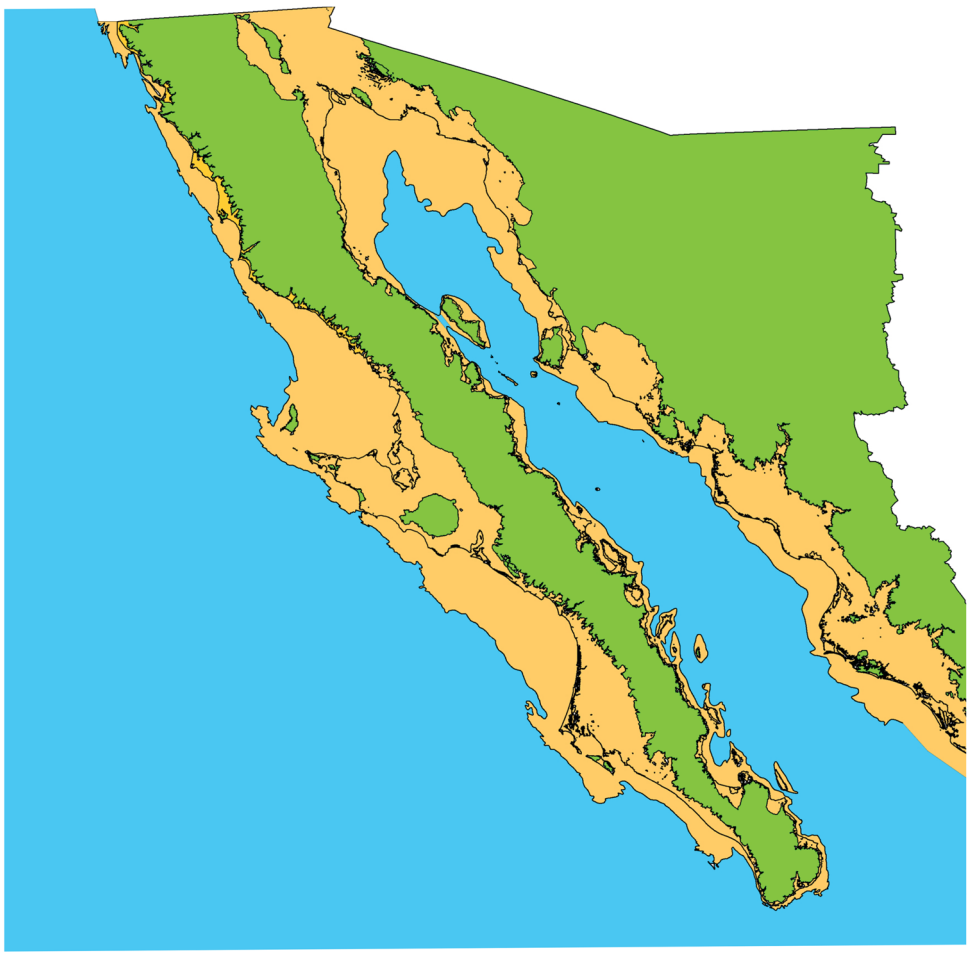

Fig. 2 Reconstruction of Gulf of California coastline during last glacial maximum during the Pleistocene epoch, using 100 m isobath as a reference. *Blue* areas represent ocean, *green* areas represent land with temperate (mesic) vegetation, and *brown* areas indicate land with arid vegetation. *Brown* areas beyond the actual coastline represent continental shelf exposed with decreased sea levels during the glacial cycle. (Color figure online)

comparisons with San Felipe, Puertecitos and Isla Tiburón (North), though p-values could not be generated for pairwise G''_{ST} values. We found no evidence for isolation by distance when we controlled for either geographic distance ($r = -0.386$, $p = 0.983$) or hierarchical population structure ($r = -0.104$, $p = 0.756$).

Both a priori approaches suggested the presence of multiple genetic groups. Two potential barriers to larval dispersal were identified in the northern Gulf using the computational geometry approach (Fig. 3). The first barrier strongly isolated San Felipe and Puertecitos on the peninsula from sites on the mainland at similar latitudes in the upper Gulf. The second barrier isolated the Bahía de los Angeles area from sites across the Gulf, and Isla Tiburón (North) and Isla Patos were most divergent from mainland sites to their north. Results from the ΔK and ΔF_{ST} methods implemented in CORRSIEVE suggested $K \geq 4$ best fit the data (Fig. S3 and Table S5). There were 5 sites where population level assignments to one of the clusters was greater than 50 percent, meaning when all samples were pooled together there was greater than 50 percent assignment to a given cluster. Two sites in the northwest Gulf were largely assigned to a specific (red) cluster—Isla San Lorenzo (0.518) and San Luis Gonzaga (0.675). Three sites in the northeast Gulf were largely assigned to the yellow cluster—Puerto Peñasco (0.568), Isla San Pedro Nolasco (0.565), and Isla San Pedro Martír (0.680) to the yellow cluster.

Migration patterns

Long-term migration rates suggest asymmetrical larval dispersal is occurring between the northern and central Gulf. Based on mtDNA, an average of 132.1 (95 %

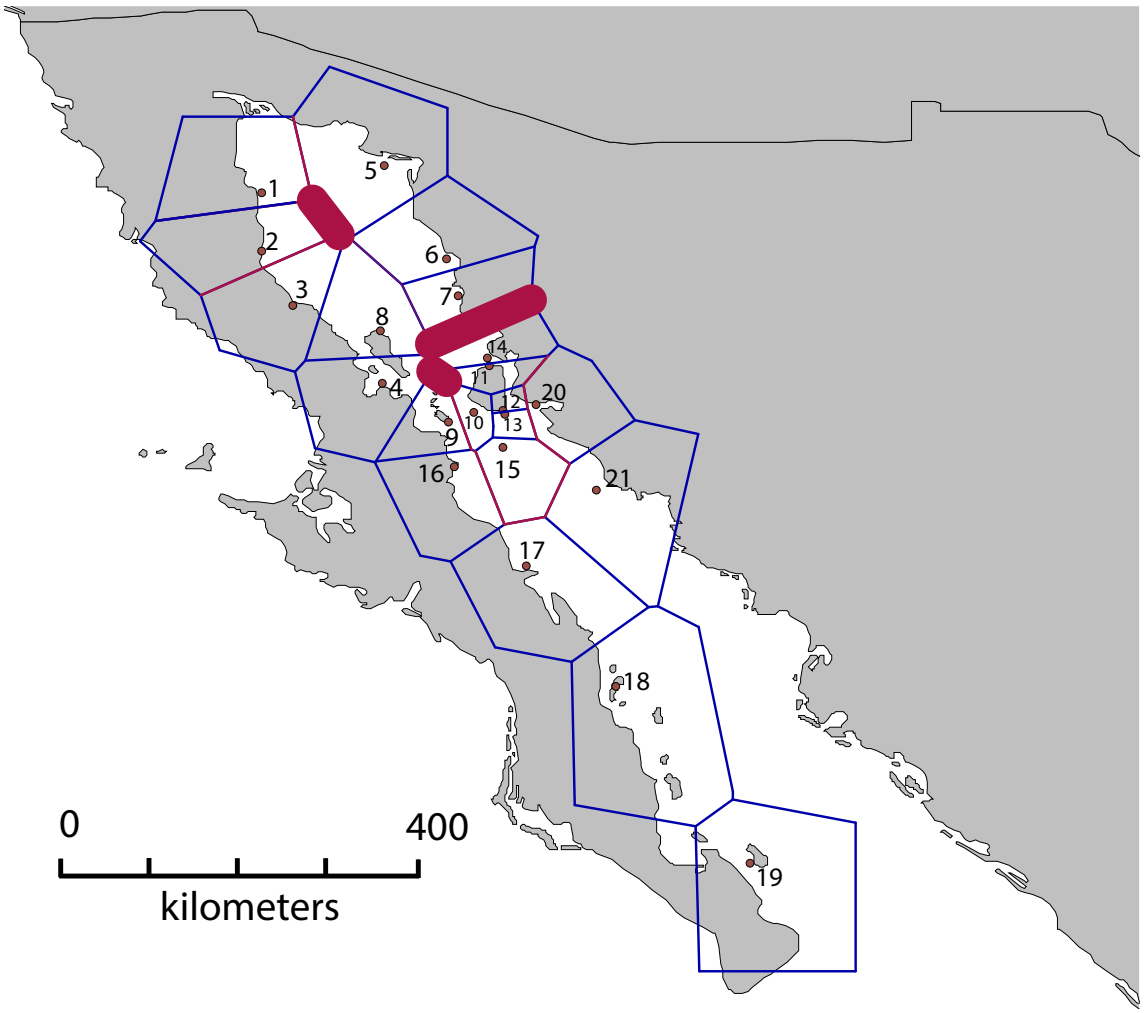

Fig. 3 Identified barriers to larval dispersal in the Gulf of California. Genetic barriers between leopard grouper subpopulations, using Delaunay triangulation and Voronoi tessellation implemented in barrier. Each sampling locality is located inside a *blue* cell, with thickness of barrier lines (in *red*) proportional to the frequency with which a given barrier is observed in replicate analyses and inversely proportional to permeability. (Color figure online)

posterior distribution [PD] = 86.5–175.0) migrants per generation move from the northern to central Gulf, while only 26.2 (95 % PD = 2.9–54.7) migrants per generation move in the opposite direction. Similarly in the microsatellite data set, an average of 581.9 (95 % PD = 390.0–750.0) migrants per generation move from the northern to central Gulf, while 220.0 (95 % PD = 72.5–320.0) migrants per generation move in the opposite direction.

Discussion

Demographic history

The majority of leopard grouper mitochondrial lineages have diverged since the last glacial maximum (LGM), when there was a dramatic drop in sea levels of 100–150 m (Fig. 2). Tajima's D values revealed evidence of population expansion in some of the northernmost subpopulations, from sampling sites located on the large expanse of shelf that would have been exposed in the upper Gulf during the LGM. In contrast, Fu's F_S values showed evidence of population expansions at all sites except Isla Ángel de la Guarda, Isla San Lorenzo, Isla Tiburón (South) and Puerto Lobos. Sites with evidence of population expansion were found along exposed shelves in the shallow upper Gulf (San Felipe, Puertecitos, Puerto Peñasco), on land-bridge islands with shallow channels connecting them to mainland (Islas Tiburón, Datil, Patos, San Marcos, Carmen, San Pedro Nolasco), and on exposed shelf in the area near Bahía Kino. Deep water surrounding Isla Ángel de la Guarda or Isla San Lorenzo in the Midriff Islands may explain why population expansion was not observed at these localities, as these deep-water sites would have remained demographically stable in the long-term despite sea level fluctuations. Evidence of population expansion in leopard grouper subpopulations is similar to patterns observed for other marine invertebrates and fishes occupying shallow waters in the Gulf of California based on Tajima's D, Fu's F_S, and divergence dates (Pfeiler et al. 2005, 2008). These observations highlight the conservation value of certain populations in the Midriff Islands region, particularly those located around deep waters that may serve as sources for new populations after periods of climate variability during the Pleistocene.

Contemporary mechanisms restricting gene flow

The magnitude of population structure observed for marine organisms in the Gulf of California ranges from little to none (Bernardi et al. 2003; Pfeiler et al. 2005) to strong (Riginos 2005). If passive movement is assumed, leopard grouper larvae have the potential to travel considerable distances (up to 300 km) during the spawning period (e.g. May–June) based on ocean current speeds (Soria et al. 2014). We found considerable genetic differentiation among distinct groups of leopard grouper in the Gulf, particularly between peninsular and mainland sites in the northern Gulf.

Trends found here in the mtDNA and microsatellite data sets confirmed patterns observed in a recent study by Munguía-Vega et al. (2014), which modeled metapopulation dynamics for leopard grouper using a biophysical oceanographic model paired with empirical genetic data. This suggests that asymmetrical oceanographic currents in the northern Gulf could explain sub-regional differences between peninsular and mainland localities. Specifically, a basin-wide counterclockwise gyre in the northern Gulf transports larvae during the spring and summer from the peninsula and Isla Ángel de la Guarda through two main routes. The first path is southward toward Isla San Lorenzo, San Esteban and Isla Tiburón (South), and then northward toward Isla Tiburón (North). Work by Munguía-Vega et al. (2014) revealed that Isla Tiburón (North) is a downstream site with large oceanographic distances with most sites from the Midriff Islands region. These large oceanographic distances from Isla Tiburón (North) are due to its high level of haplotypic diversity, a result of the direction of the predominant flow of currents contributing larvae from multiple upstream sources to this site. The second path is northward directly towards Puerto Libertad.

Additionally, oceanographic currents provided evidence of larval retention, particularly in the region around Isla Tiburón (North) and Puerto Libertad (Munguía-Vega et al. 2014), where a genetic barrier was observed in our study. Recent work by Iacchei et al. (2013) suggests that high genetic differentiation in sites may be due to localized larval retention. Here by local retention we mean the proportion of larvae produced at a site that are retained at the end of the PLD (Burgess et al. 2013). Thus, the presence of a barrier around the eastern Midriff Islands suggests both local retention of larvae and that larvae may be transported along multiple paths between sampling localities. The presence of a genetic barrier in the upper Gulf of California could similarly be explained by oceanographic phenomena that facilitate retention of larvae. Populations along the mainland coast of Sonora are linked by a northward current, divided from populations on the Baja peninsula (which are linked by a southward current) by a region of high local retention in the upper Gulf (Marinone 2012; Munguía-Vega et al. In press).

Asymmetric migration observed in leopard grouper, with disproportionately more genetic exchange from the northern to the central Gulf, has been observed in another epinephelid fish species (Riginos 2005). Three-dimensional modeling of

circulation in the Gulf of California can explain this trend (Marinone 2003), as surface currents are predominately moving from north to south in May along the peninsular coastline when leopard grouper larvae would be in the water column (Erisman et al. 2007). Thus, the predominant direction of migration events from north to south might be explained by the fact that the majority of localities in the central Gulf sampled in our study were located along the peninsula and not along the mainland side of the Gulf.

Habitat distribution, adult movement patterns, and temporal sampling are alternative factors that might also be driving geographic patterns of genetic variation observed in leopard grouper. Discontinuity of suitable habitat can facilitate reduced gene flow among populations (Johansson et al. 2008; Fraser et al. 2010; Milana et al. 2012). A gap in rocky reef habitat between Puerto Peñasco (mainland) and Puertecitos (peninsula) in the shallow upper Gulf is present (Turk-Boyer et al. 2014), potentially contributing to genetic differentiation. Additionally, movement patterns of some adult groupers indicate they may be less likely to migrate across deep channels (Colin et al. 1987; Starr et al. 2007). However, this behavior may not be applicable to leopard grouper based on a recent, small-scale study that showed adults moving across a deep channel (approx. 1.5 km) between Espiritu Santo and Marisla Seamount (separated by approx. 20 km) in the southern Gulf (Tinhan et al. 2014). Whether this trend holds in other regions in the Gulf of California will determine the relative importance of adult movement in either facilitating or inhibiting connectivity among coastal and island spawning stocks. Finally, given samples were acquired over a 6-year period, we considered the effect of temporal sampling on observed population structure. However, given the average age at sexual maturity for leopard grouper is 3–4 years and fish may reach an age of 21 years or more (Diaz-Uribe et al. 2001; Erisman et al. 2007), we believe that temporal sampling is an unlikely explanation for observed patterns of genetic differentiation. In this study, the sampling period is potentially only about two generations.

Relevance for conservation and fisheries management

According to the Carta Nacional Pesquera, approximately 60 % of fisheries in the Gulf of California are either at capacity or overexploited (Cisneros-Mata 2010). Deteriorating conditions in the Gulf of California's fisheries resources warrant an improvement of conservation and fisheries management efforts. Developing an effective management plan for leopard grouper would ensure the long-term sustainability of a profitable fishery for communities highly dependent on fishing-derived income (Cinti et al. 2009), while preserving the evolutionary potential of leopard grouper populations.

Considering the evidence of sub-regional genetic differentiation and asymmetrical gene flow between the northern and central Gulf of California, marine reserves may be an effective fisheries management tool for leopard grouper. Prime locations to establish no-take zones would be in the areas around Isla San Lorenzo, Isla San Esteban, Isla Tiburón (North), and Isla Patos, based on high levels of genetic diversity and barriers to larval dispersal observed. These no-take zones could provide a source of larvae to heavily fished grounds on the mainland coast north of Isla Tiburón. Both fishing intensity and diversity of targeted species are high in the Midriff Islands relative to other areas in the northern Gulf, particularly in the region around Isla Tiburón (Moreno-Báez 2010). Work by Munguía-Vega et al. (2014) also revealed that fishing in this region is sustained due to high levels of local retention, in part due to oceanographic patterns in the northern Gulf that concentrate leopard grouper larvae from throughout the northern Gulf. In our study, demographic analyses also identified subpopulations with varying evolutionary histories—those that experienced potential population extinction during the Pleistocene, and those whose population sizes have not fluctuated over time (east of Isla Ángel de la Guarda and Isla San Lorenzo). These deep-water leopard grouper subpopulations that have been stable over time, which serve as key sources that directly supply larvae to fishing grounds near Puerto Libertad and the upper Gulf, could benefit from larger or better enforced no-take zones within present marine reserves. As a long-term management strategy for leopard grouper, such no-take zones should consider conserving both the unique genetic diversity and demographic histories of these subpopulations.

The new no-take zones proposed here could be integrated with existing national parks and reserves in the Gulf of California to form a well-connected network that would help preserve the evolutionary potential of leopard grouper subpopulations (i.e., genetic diversity), as well as protect important sources of larvae. Observed high levels of genetic connectivity among sites in our study suggest that, if these new no-take reserves are located upstream, they could form an effective network of marine reserves across the Gulf of California that could provide overflow of larvae to adjacent, downstream overfished areas. However, given the direction of the oceanographic gyres and coastal currents completely reverse during fall and winter (Marinone 2012; Munguía-Vega et al. In press), it is expected that optimal placement of new no-take zones would vary for species spawning October to February. Additionally, the ultimate effectiveness of these networks will be highly contingent upon enforcement, governance, and increased community involvement to ensure that regulations and boundaries of no-take zones are respected (Rife et al. 2013).

Conclusion

We observed genetically differentiated units of leopard grouper within the Gulf of California, with divergence of mitochondrial lineages dating to the late Pleistocene. Observed patterns of genetic differentiation are likely attributed to asymmetrical oceanographic currents. Based on our genetic findings and current fishing pressure in certain regions in the Gulf, we recommend implementing small no-take zones in the areas east of Isla Ángel de la Guarda, around Isla San Lorenzo and Isla San Esteban, and north of Isla Tiburón. These new no-take zones could link to current marine reserves distributed throughout the Gulf of California, in order to more effectively protect and replenish subpopulations of commercially exploited leopard grouper.

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