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Genetic signature of a recent metapopulation bottleneck in the olive ridley turtle (*Lepidochelys olivacea*) after intensive commercial exploitation in Mexico

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ABSTRACT

Information on the demographic and genetic consequences of overexploitation of large marine vertebrates is often difficult to demonstrate on ecological time scales. We investigate the genetic impacts of recent commercial activities along Mexico's Pacific coast on the nesting colonies of a long-lived vertebrate of conservation concern, the olive ridley turtle (*Lepidochelys olivacea*). This species was severely impacted by a commercial fishery between 1960 and 1990 (e.g. >350,000 individuals were caught in a single year), depleting important nesting areas within few decades. Microsatellite DNA variation of 334 samples representing 18 nesting sites revealed a clear signature of recent bottlenecks associated with changes in allelic diversity. Consistent with theoretical expectations and other empirical studies, we found no evidence for bottlenecks based on measures of heterozygosity. The bottleneck signal was strong across the highly connected metapopulation and also apparent in six nesting sites in a pattern consistent with the history of demographic disequilibria produced by their overexploitation. In addition, we clarify population structure across Mexico and show that Mismaloya, a key colony where human harvest led to a dramatic change in the species' reproduction mode, has not been supplemented by gene flow after the bottleneck and has diverged genetically from other demes. This is perhaps the first study to detect recent signatures of anthropogenic-driven population declines in sea turtles using genetics. This enables managers to consider information about genetic signatures of contemporary demographic changes during the development of conservation management plans and during population monitoring.

1. Introduction

Pressures imposed by overharvesting of wildlife have accounted for the decline of several populations over the last century and have placed many others at high risk of extinction (Hutchings, 2000; Larson et al., 2002). Such changes in effective population size (*Ne*), known as population bottlenecks, are usually accompanied by reductions in genetic diversity. The identification of recent genetic bottlenecks (i.e. during ecological timescales) in species of conservation concern can provide a framework for enhancing management practices directed to restoring metapopulation connectivity and minimizing further loss of genetic variability and fitness (e.g. Taylor et al., 1997; Reed and Frankham, 2003; Shama et al., 2011). In marine ecosystems, the lack of systematic broad-scale inventories and baseline data makes it particularly difficult to assess the impacts of humans on the decline of marine populations (Edgar et al., 2005). Furthermore, genetic signatures of recent demographic collapses are hard to demonstrate in marine vertebrates targeted by commercial activities because these species usually have long generation times, relatively large pre-exploitation *Ne* and moderate to high connectivity – features that should buffer processes driving the loss of genetic variability (e.g. Busch et al., 2007; Hailer et al., 2006). Therefore, information is generally not available for resource managers and thus hardly incorporated into management plans, a problem particularly germane in developing countries (Allendorf et al., 2008; Harris et al., 2002).

Here we investigate the genetic consequences of a recent, large-scale commercial exploitation in a long-lived marine vertebrate, the olive ridley turtle (*Lepidochelys olivacea*). These turtles exhibit two modes of reproduction that define nesting areas as either solitary or *arribada* sites. Solitary nesting, the most common mode, takes place when individual females emerge to lay eggs at low densities with no apparent synchronicity between individual events. On the other hand, the less common *arribada* mode, consists of large numbers of females emerging synchronously over relatively

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short intervals (2–7 days) to nest at very high densities. This reproductive strategy is unique to the genus *Lepidochelys* and occurs annually in a few places worldwide, with seasonal variability between regions (Bernardo and Plotkin, 2007). During the 1960s, nesting females and eggs of olive ridleys were severely harvested by humans along the Pacific coast of Mexico. The extensive industrial harvest depleted important nesting colonies of olive ridleys within a few decades (Márquez-M, 1996). For instance, 14,000 tons (~350,000 individuals) were caught in a single year (1968), but it has been suggested that statistics were underestimated by an order of magnitude (Márquez-M et al., 1982). Commercial exploitation continued thorough the 80s and depleted several massive nesting colonies in Mexico where *arribadas* no longer occur (e.g. Mismaloya Beach) and almost collapsed other *arribada* colonies such as Escobilla Beach (in Oaxaca). Eventually, the industrial harvest was officially banned in 1990 (Diario Oficial de la Federacion, DOF-1990) and since then demographic recoveries were observed for a few nesting sites, especially in Escobilla (Márquez-M et al., 2007). Although a recent global population assessment of olive ridleys (Abreu-Grobois and Plotkin, 2008) resulted in a change of its IUCN Red List classification from 'Endangered' to 'Vulnerable', the breeding population in the Mexican Pacific is still classified as 'Endangered' by the U.S. Endangered Species Act (ESA). Moreover, long-term data for other areas remain limited, and illegal egg harvest and bycatch in shrimp trawls are still significant threats (Wallace et al., 2010). In addition, genetic findings suggest the existence of a genetically divergent and less diverse population in the Baja California Peninsula, Mexico (mtDNA $\Phi_{ST} = 0.048$, $P = 0.006$) (López-Castro and Rocha-Olivares, 2005). These results imply that the contribution of small solitary nesting beaches to overall population structure and diversity in olive ridleys might have been underestimated by previous studies (Briseño-Dueñas, 1998; López-Chávez, 2000; Bowen and Karl, 2007).

The recorded history of massive harvesting of olive ridleys across several colonies in Mexico (Márquez-M et al., 1982) provides a unique opportunity to test for genetic signatures of recent anthropogenic-driven demographic collapses in a marine vertebrate and to identify nesting colonies that might have declined but for which little ecological information exist. In sea turtles, genetic bottlenecks have been linked to historic human activities and environmental changes that took place over the last 2000 years (Plot et al., 2012). However studies that explored demographic reductions over recent time scales failed to detect genetic evidence for bottlenecks (Carreras et al., 2007). Assessment of bottlenecks in olive ridleys at the levels of both the deme and the regional nesting area enables the identification of links between nesting colony exploitation and metapopulation dynamics and the evaluation of recovery trends associated with conservation policies and practices in sea turtles. In addition, Mexico holds one of the world's largest nesting colonies of the species (Escobilla Beach), considered by some as a reservoir of genetic variability and by others as a valuable resource for humans (Campbell, 1998).

In this study we generated what is arguably the largest microsatellite DNA dataset for a nesting geographic area of a sea turtle species (i.e. 334 individuals from 18 nesting sites) to (i) clarify fine-scale population structure of olive ridleys along Mexico's Pacific coast and (ii) test for bottlenecks potentially linked to the recent history of commercial exploitation. During the process of achieving these aims we explore two popular statistical approaches of bottleneck detection: heterozygosity excess (Cornuet and Luikart, 1996) and the *M*-ratio (Garza and Williamson, 2001). These approaches are expected to show differential sensitivity to detect signal associated with transient population reductions, such as the one recorded for our system. Our study has broader implications to genetic surveys of demographic variation in marine vertebrates targeted by commercial activities. It is perhaps the first study to detect recent genetic signatures of anthropogenic-driven declines in sea turtles and to show that localized exploitation (i.e. at the nesting site) can have genetic consequences across the entire regional metapopulation (i.e. along Mexico's Pacific coast).

2. Materials and methods

2.1. Sample collection

We sampled solitary and *arribada* nesting beaches of olive ridley in Mexico (18 sites) during 2006 (Fig. 1). Categories of nesting beaches are detailed in Table 1. Skin biopsies were collected from tagged nesting females to avoid replication of samples (FitzSimmons et al., 2000) and tissue was preserved using 20% DMSO/saturated NaCl solution. In areas where sampling of females was difficult, tissue from one dead hatchling per nest was taken within the 15-day inter-nesting period. Genetic analyses are based on 13 nesting areas as beaches with less than 15 samples were assigned to major nesting areas using the criterion of the geographically closest neighbour.

2.2. DNA purification, amplification and genotyping

We extracted DNA using a modified salting-out protocol (Sunnucks and Hales, 1996) by increasing volumes on digestion (600 µl of TNES) and precipitation (170 µl NaCl) steps. A total of 334 samples were genotyped at 10 microsatellite loci (OR1, OR2, OR4, OR7, OR9, OR11, OR14, OR16, OR20 and OR22) (Aggarwal et al., 2008, 2004). Touchdown PCR profile consisted of 3 min at 94 °C followed by 35 cycles (94 °C/20 s; 61 °C down to 53 °C until fifth cycle/45 s; 72 °C/1 min), and 10 min at 72 °C. Touchdown for locus OR20 was modified to 57-53 °C. Amplification reactions contained: ~5–10 ng DNA, 1xMango taq reaction buffer (Bioline), 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.4 mM each primer, 0.5 U Mango-Taq DNA Polymerase (Bioline), 0.1 mM fluorescently labelled M13 primer. Allele separation was performed on an ABI 3730 (Applied Biosystems Inc, CA) and genotypes scored on GENEMAPPER™ 4.0 (Applied Biosystems). Null alleles and large allele dropout were assessed in MICRO-CHECKER (Oosterhout et al., 2003).

2.3. Genetic diversity and detection of bottlenecks

Departures from Hardy–Weinberg expectations (HWE) and linkage disequilibrium (LD) among loci were tested in GENEPOP v 4.0 (Rousset, 2008), and significance adjusted with sequential Bonferroni correction. Expected (*He*) and observed (*Ho*) heterozygosity, allelic richness (AR) and F_{IS} were estimated using FSTAT 2.9.3 (Goudet, 2001).

Recent genetic bottlenecks were assessed using two distinct analytical approaches: heterozygosity excess (Cornuet and Luikart, 1996) and the ratio (*M*) of the total number of alleles (*k*) to the range in allele sizes (*r*), or *M*-ratio (Garza and Williamson, 2001). Both tests compare observed results to theoretical expectations based on a population at equilibrium. The two approaches were used to explore our data using the same sets of parameter conditions and mutational models. Variation encompassed the values for P_g (the proportion of multi-step mutations) and δ_g (the mean size of multi-step mutations) according to estimates for olive ridley turtles ($P_g = 0.27$, $\delta_g = 3.3$; Hoekert et al., 2002), green turtles ($P_g = 0.57$, $\delta_g = 4.0$; FitzSimmons, 1998), widely used parameters ($P_g = 0.10$, $\delta_g = 3.1$; Piry et al., 1999, and $P_g = 0.10$, $\delta_g = 3.5$; Garza and Williamson, 2001) and values recently proposed as suitable for assessing population bottlenecks ($P_g = 0.22$, $\delta_g = 3.1$; Peery et al., 2012). Additionally, we explored conservative scenarios by gradually increasing the proportion of multi-step mutations by 0.05 units up to

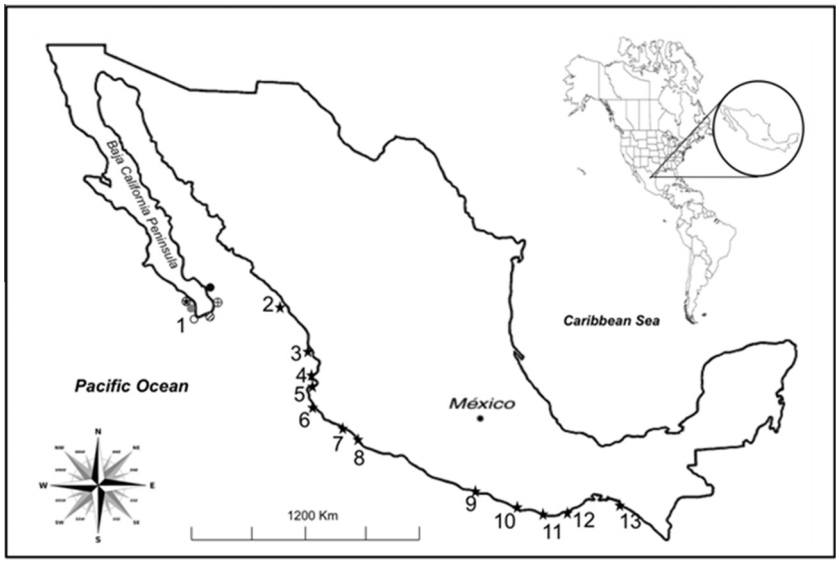

Fig. 1. Sampling sites of olive ridleys in Mexico. (1) Baja California Peninsula: (star) Todos Santos, (grey dot) Pescadero, (white dot) San Cristobal, (striped dot), San José del Cabo, (crossed dot) Cabo Pulmo, (black dot) Punta Colorada and Punta Arenas. In the continent: (2) El Verde, (3) Platanitos, (4) Nuevo Vallarta, (5) Puerto Vallarta-La Gloria, (6) Mismaloya, (7) Boca de Apiza, (8) Playa Ticuiz, (9) Tierra Colorada, (10) San Juan de Chacahua, (11) Escobilla, (12) Barra de la Cruz and (13) Puerto Arista.

Table 1
Categories of olive ridley nesting areas in Mexico. *Sources*: (1) Convención Interamericana de Tortugas Marinas (2012), (2) Programa nacional de protección, conservación, investigación y manejo de tortugas marinas (2000), (3) Lopéz-Castro et al. (2004), (4) SEMARNAT report 2001–2005 in Abreu-Grobois and Plotkin (2008).

State	Locality	Category	Number of nests[a]	Source
Baja	Todos Santos	S	NR	–
California	Pescadero	S	NR	–
Sur	San Cristóbal	S	NR	–
	El Suspiro	S	101–500	1
	San Jose del cabo	S	NR	–
	Cabo Pulmo	S	55	3
	Punta Arenas	S	NR	–
	Punta Colorada	S	NR	–
Sinaloa	El Verde	S	1607	1
			523	2
			1160	4
Nayarit	Platanitos	S	1001–5000	1
			424/439	2
			1301	4
	Nuevo Vallarta	S	1001–5000	1
Jalisco	Puerto Vallarta/Las Glorias	S	NR	–
	Mismaloya	S	232/251	2
			2328	4
Colima	Boca de Apiza	S	1001–5000	1
			458	2
Michoacan	Playa Ticuiz	S	NR	–
Guerrero	Tierra Colorada	S	868	1
Oaxaca	Escobilla	A	1,502,393	1
			248,063/956 108	2
	Barra de la Cruz	S	662	1
			67	2
	San Juan de Chacahua	S	501–1000	1
			717/3944	2
			2042	4
Chiapas	Punta Arista	S	3924	1
			137	2
			707	4

[a] Annual number of nests. (S) Solitary sites, (A) Arribada sites, (NR) not reported.

$P_g = 0.57$, using a constant value of $\delta_g = 3.1$. Data were explored at two levels: (1) whole population and (2) deme (nesting colony), since the latter is of management interest. Mainland nesting colonies were also tested independently from peninsular nesting sites.

To assess the significance of heterozygosity excess we used a Wilcoxon sign-rank test based on a two-phase mutation model and a qualitative descriptor of allele frequency distribution (mode-shift indicator), both implemented in BOTTLENECK v 1.2.02 (Cornuet and Luikart, 1996). The variance of the size of multi-step mutations was estimated following the equations proposed by Williamson-Natesan, 2005). For the *M*-ratio, we estimated empirical *M*-value and *M* critical. The value of *M* decreases after a population is reduced in size with magnitude correlated with the severity and duration of the bottleneck (Garza and Williamson, 2001). The empirical *M*-value was compared to a simulated distribution of values (*M* critical) to assess significance based on 10,000

simulations. The *M* critical is set to the lower 5% tail of the distribution, below which it is assumed that observed ratios are from a population that has experienced a significant reduction in size. We obtained values using the parameters of theta ($4 * (\text{historical}) N_e * \text{mutation rate } \mu$), δ_g and p_s (as described above), a mutation rate of 5.7×10^{-4} for microsatellites (FitzSimmons, 1998) and a wide range of pre-bottlenecked N_e of 1000, 2000, 4000 and 6000 and 50,000. Additionally, historical values of N_e were used using two long-term estimators proposed by Hartl and Clark (1989) and Ohta and Kimuraa (1973) based on the infinite allele (IAM) and step-wise (SMM) mutation models, respectively: $N_e = H_E/4\mu(1 - H_E)$ and $N_e = (1/(1 - H_E))^2 - 1/8\mu$. Our estimates of N_e varied from 1112.9 to 2038.1 (IAM) and from 2351.5 to 6191.8 (SMM). To assess statistical power for inferring bottlenecks under our study conditions (i.e. number of samples and loci), we simulated scenarios incorporating liberal and conservative pre-bottleneck N_e for both *arribada* and solitary nesting sites. In addition, we simulated power of inference for the metapopulation using information from large documented population reductions, such as the one that took place in the *arribada* site of Mismaloya (Abreu-Grobois and Plotkin, 2008; Márquez-M et al., 1982). We set additional parameters to $P_g = 0.22$, mutation rate = 5.7×10^{-4}, and generation time to 20 years. A non-recovery simulation model was used because most of the sampled nesting colonies had their historic population size reduced to low numbers and have not shown substantial recovery. We implemented 1000 replicates to estimate the statistical power of the data set using SPOTG (Hoban et al., 2013).

2.4. Analysis of spatial population structure

Population subdivision considering both global and pairwise Wright's F_{ST} was tested for significance using ARLEQUIN 3.11 (Excoffier et al., 2005), and adjusted with Bonferroni sequential correction. We also calculated Jost' D_{EST} estimate (10,000 permutations) in GENALEX 6.5 (Peakall and Smouse, 2012) for comparison, as F_{ST} may not accurately measure the magnitude of genetic differentiation under low divergence and high heterozygosity (Heller and Siegismund, 2009). A hierarchical analysis of molecular variance (AMOVA) among colonies at Baja California Peninsula (4; $n = 80$) and those in mainland (12; $n = 285$) was carried out in ARLEQUIN.

We examined relationships between genetic differentiation and geographic distance at various spatial scales calculating autocorrelation coefficients of multilocus genotypes (r) among individuals sampled in the same locality (distance class 0) and among individuals separated by 100 km up to 1800 km in GENALEX. A broader test of isolation by distance in IBDWS 3.16 (Jensen et al., 2005) using both F_{ST} and D_{EST} was also carried out. Geographic distances corresponded to the shortest possible sea distance between nesting sites estimated in GOOGLE EARTH.

We further tested population subdivision using a Bayesian model-based clustering analysis in STRUCTURE 2.1 (Pritchard et al., 2000). Two admixture models were tested: standard and LocPrior models, with the latter model designed to detect weak population structure. The identification of populations or *K*-clusters followed the method of Evanno et al., 2005), with ten independent runs for each of $K = 1$–13 using 1×10^5 MCMC iterations after a burn-in of 1×10^4, as results did not change with longer runs.

3. Results

3.1. Genetic variation and bottlenecks

No deviations from HWE or evidence of LD were detected in our data. Null alleles were identified for one locus (OR2) at only six out of 18 nesting areas. This locus was included in subsequent analyses since results remained unchanged if removed from the data set. All microsatellite loci were variable, with an average of 10.5 alleles per locus, mean observed heterozygosity of 0.76 and allelic richness of 6.18 (Table 2, online appendix: Table A1).

A strong signal associated with population bottlenecks was detected for olive ridley turtles in Mexico based on the *M*-ratio test, a result observed across a wide range of mutational models and theta values (Table 3). Observed *M*-values suggestive of bottlenecks (i.e. lower than the estimated *M* critical values and the *M* of 0.70 associated with populations known to have undergone recent bottlenecks (Garza and Williamson, 2001) varied between 0.59 and 0.77. These statistically significant results were consistently observed in six nesting areas: Baja California Peninsula, Mismaloya, Playa Ticuiz, Boca de Apiza, Barra de la Cruz and Puerto Arista. Remarkably, the signal of genetic bottleneck also remained strong when *M*-ratio was estimated across all Mexican nesting colonies pooled as a single population ($n = 334$) or when pooling all mainland colonies ($n = 258$; after excluding peninsular Baja California). When testing more conservative scenarios (i.e. $P_g \geqslant 0.32$) at the deme level, the signal of bottlenecks remained significant for the nesting colonies of Baja California Peninsula and Playa Ticuiz (Table 3, online appendix: Table A2). In marked contrast, results from the test based on excess of heterozygotes did not provide evidence for genetic bottlenecks ($P = 0.99$; $P = 0.98$ and normal L-shift distribution; online appendix: Table A3), regardless if nesting colonies were analyzed separately or pooled.

Simulation analyses indicated a fairly good power (79.7%) to correctly reject the null hypothesis of no bottlenecks for the entire metapopulation. In general, simulations indicated probabilities decreasing with smaller sample sizes and they resulted generally in reduced power either for *arribada* or solitary sites regarding of the scenarios tested (up to 17.5% and 12.9%; online appendix: Fig. A2).

3.2. Population differentiation

Olive ridleys in Mexico showed very low levels of differentiation and no clear geographic pattern of population structure. The hypothesis of random mating across the vast sampled area in the Pacific coast could not be rejected when all colonies were pooled together ($P = 1.000$). Overall, most pairwise nesting comparisons were non-significant for both F_{ST} and D_{EST}, except for nesting colonies of Mismaloya, San Juan de Chacahua and Puerto Arista which showed low but statistically significant differentiation (Table 4). Hierarchical AMOVA indicated no significant structure between colonies on Baja California Peninsula and those from mainland ($F_{ST} = -0.0004$, $P = 0.595$). No signal of isolation by geographic distance was detected ($r = 0.027$, $P = 0.351$). However, as expected due to the natal homing behavior of the species, positive spatial clustering of genotypically similar individuals was observed at a nesting colony level (distance class = 0 km; $r = 0.008$, $P = 0.001$, Fig. 2), but not over larger distance intervals. Although the Bayesian analysis of structure suggested $K = 2$ as the most likely number of populations, the vast majority of individuals across all nesting colonies show mixed coancestry. Nonetheless, some of the nesting sites such as Mismaloya show a greater membership to one population only (black bars in online appendix: Fig. A1).

4. Discussion

We disclose genetic signal associated with recent and brief (around 1.5 generations) human-driven population bottlenecks in olive ridleys from the Mexican Pacific coast. The results indicate that the intensive harvesting of sea turtles between 1960 and late

Table 2
Summary statistics of genetic diversity based on ten microsatellite markers for 13 nesting areas of olive ridleys in Mexico.

Region	Collection site		Sample size	N_A	Ho	He	AR	F_{IS}
Baja California Sur	Todos Santos, Pescadero, San Cristobal, San José del Cabo, Cabo Pulmo, Punta Colorada, Punta Arenas.	(BCP)	80	15.3	0.738	0.803	6.436	0.087
Sinaloa	El Verde	(EVE)	18	9.4	0.658	0.732	5.957	0.13
Nayarit	Platanitos	(PLA)	21	11.1	0.770	0.789	6.519	0.049
	Nuevo Vallarta	(NVA)	20	9.7	0.598	0.757	5.928	0.237
Jalisco	PuertoVallarta / La Gloria	(PVG)	25	11.3	0.703	0.690	6.098	0.103
	Mismaloya	(MIS)	25	9.2	0.626	0.690	5.610	0.118
Michoacan	Ticuiz	(PTI)	15	10	0.781	0.781	6.491	0.04
Colima	Boca de Apiza	(BAP)	21	10.7	0.743	0.779	6.426	0.074
Guerrero	Tierra Colorada	(TCO)	18	10.2	0.712	0.776	6.390	0.114
Oaxaca	San Juan de Chacahua	(SJC)	30	11.5	0.738	0.777	6.194	0.068
	Barra de la Cruz	(BCR)	24	9.8	0.697	0.795	6.116	0.151
	Escobilla	(ESC)	40	12.7	0.737	0.801	6.434	0.094
Chiapas	Puerto Arista	(PAR)	28	10.7	0.650	0.743	5.856	0.148

(NA) number of alleles, (Ho) observed heterozygosity, (He) expected heterozygosity, (AR) allelic richness, (F_{IS}) coefficient of inbreeding.

1980s at the deme level (i.e. nesting beaches) has caused genetic erosion of the metapopulation found along the Mexico's Pacific coast – a conclusion supported with good statistical power. In addition, genetic bottlenecks were in some cases also identified (albeit with low statistical power) for colonies where historical records were not available. We demonstrate the importance of genotyping a large sample collected across a vast nesting geographic region that includes both commercially exploited and non-exploited demes and the exploration of statistical tests that encompass a range of population parameters for genetic assessments of bottlenecks in a marine vertebrate. Our study showed that recent and localized anthropogenic harvest has an effect in the genetic diversity of a sea turtle metapopulation. This enables managers to consider information about genetic signatures of contemporary demographic changes during both the development of conservation management plans and during population monitoring.

4.1. Genetic diversity and the effect of commercial fishery

Pre-exploitation levels of genetic diversity in olive ridleys from Mexico are unknown. Our estimates indicated high levels of genetic diversity for the species in Mexico's Pacific coast (mean H_e varied between 0.69 and 0.80 across the 13 nesting sites; Table 2). Diversity levels were similar to olive ridleys from nesting areas in the broader region, such as Costa Rica (mean H_e varied from 0.78 to 0.94 across three nesting sites) (Jensen et al., 2006), and generally higher than in a depleted population from the Atlantic Ocean (mean H_e was 0.61 based on two sites) (Plot et al., 2012). Reductions of genetic diversity associated with population bottlenecks are typically slow and normally detected after prolonged and intensive demographic collapses (e.g. Beheregaray et al., 2003). During early stages of bottlenecks, populations may still contain substantial heterozygosity showing only distortions in the distribution of allele frequencies, with distortions being transient and likely detectable for only a few dozen generations (Luikart et al., 1998). Our analyses detected loss of allelic diversity but not heterozygosity in olive ridleys (Table 3, online appendix: Table A3) suggestive of a recent bottleneck after three decades of over-exploitation. This represents only 1.5 discrete generation (20 years average age of parents; Abreu-Grobois and Plotkin, 2008), but is consistent with the severity of the large-scale commercial exploitation of olive ridleys in Mexico (e.g. ~350,000 individuals caught in a single year; Márquez-M et al., 1982).

The detected bottlenecks are indicative of changes in the genetic composition of olive ridleys in Mexico due to intense anthropogenic harvest. Stock collapses in populations under intensive fishing pressure, such as the North Sea cod (*Gadus morhua*), resulted in marked reductions of genetic diversity and changes in population structure with implications for subsequent recovery and adaptive potential (Hutchinson et al., 2003). In our study, the genetic results are consistent with available historic records of colonies heavily impacted by the commercial fishery in Mexico. This was particularly true for the genetically bottlenecked colony in Mismaloya (Table 3), where a higher concentration of individuals led to major harvesting efforts. In this beach, estimated changes of population size over time revealed a 99% reduction of the number of nesting females (Abreu-Grobois and Plotkin, 2008). The severe decline may have compromised the recovery of this important nesting colony, even under a scenario of moderate to high connectivity (details below) and currently increasing levels of beach protection. In fact, recent nesting activity on Mismaloya – a nesting site formerly characterized by an *arribada* mode of reproduction, remains to levels of solitary sites (Abreu-Grobois and Plotkin, 2008; Table 1).

It is also known that the fishery in Mexico relied on the contribution of solitary nesting areas to supply the market (Márquez-M et al., 1982). Our results reflect this fishing effort by identifying bottlenecks in the solitary nesting areas of Baja California Peninsula, Playa Ticuiz, Boca de Apiza, Barra de la Cruz and Puerto Arista. Importantly, demographic reductions were also suggested for colonies such as Playa Ticuiz and Baja California Peninsula, areas with very limited ecological data. However, some over-exploited nesting colonies did not show signs of population bottlenecks. This might be related to a combination of factors, such as the short span of the fishery, the success of corrective actions, and the N_e pre and after decline (N_e would be higher in *arribada* nesting sites). For instance, the *arribada* colonies of Mismaloya and San Juan de Chacahua are thought to have similar pre exploitation *Ne* to Escobilla (Abreu-Grobois and Plotkin, 2008), but so far have not responded to increased protection. It is also plausible that the modest size of our deme samples and the number of loci used influenced the probability of bottleneck detection in some nesting colonies. Theoretical simulations have shown that this probability decreases with less markers and individuals sampled (Peery et al., 2012). The power analysis explored here followed a similar trend, showing reduced power when inferring population bottlenecks at deme level. However, some nesting colonies with comparable bottleneck detection probability (i.e. Mismaloya and Puerto Vallarta/La Gloria) derived different conclusions about evidence of bottlenecks that seems to agree with the expectations from demographic data of exploitation in Mexico. Overall, our study provides a good indication of the genetic effects of recent exploitation on the entire olive ridley metapopulation in Mexico. Finally, other biological factors

Table 3
Results of significant tests of genetic bottlenecks based on the *M*-ratio for olive ridley turtles in Mexico. Values are shown across a range of parameter conditions and mutational models for both the entire population and subpopulation (nesting colonies) levels.

Nesting area	N_e	Θ	*M*-ratio	M_C $P_g = 0.10$ $\delta_g = 3.5$	M_C $P_g = 0.10$ $\delta_g = 3.1$	M_C $P_g = 0.22$ $\delta_g = 3.1$	M_C $P_g = 0.27$ $\delta_g = 3.3$	M_C $P_g = 0.32$ $\delta_g = 3.1$	M_C $P_g = 0.37$ $\delta_g = 3.1$
Entire population ($n = 334$)	1000	2	0.632	**0.759**	**0.798**	**0.722**	**0.679**	**0.689**	**0.673**
	2000	4	0.632	**0.749**	**0.789**	**0.732**	**0.690**	**0.706**	**0.696**
	4000	8	0.632	**0.750**	**0.793**	**0.745**	**0.711**	**0.729**	**0.718**
	6000	12	0.632	**0.756**	**0.795**	**0.754**	**0.723**	**0.737**	**0.729**
	50,000	100	0.632	**0.785**	**0.798**	**0.766**	**0.745**	**0.750**	**0.744**
	IAM	4.0	0.632	**0.749**	**0.793**	**0.732**	**0.690**	**0.706**	**0.696**
	SMM	11.8	0.632	**0.757**	**0.796**	**0.756**	**0.722**	**0.737**	**0.730**
Mainland population ($n = 258$)	1000	2	0.684	**0.758**	**0.796**	**0.722**	0.675	**0.685**	0.668
	2000	4	0.684	**0.746**	**0.788**	**0.727**	**0.687**	**0.701**	**0.689**
	4000	8	0.684	**0.747**	**0.789**	**0.741**	**0.702**	**0.720**	**0.713**
	6000	12	0.684	**0.751**	**0.791**	**0.746**	**0.715**	**0.727**	**0.722**
	50,000	100	0.684	**0.762**	**0.787**	**0.755**	**0.729**	**0.736**	**0.796**
	IAM	3.9	0.684	**0.746**	**0.790**	**0.728**	**0.685**	**0.698**	**0.772**
	SMM	11.4	0.684	**0.789**	**0.791**	**0.749**	**0.714**	**0.725**	**0.719**
Baja California Peninsula ($n = 80$)	1000	2	0.658	**0.751**	**0.784**	**0.702**	0.655	**0.660**	0.641
	2000	4	0.658	**0.731**	**0.770**	**0.700**	0.654	**0.666**	0.650
	4000	8	0.658	**0.720**	**0.759**	**0.700**	**0.660**	**0.671**	**0.660**
	6000	12	0.658	**0.716**	**0.755**	**0.700**	**0.664**	**0.675**	**0.663**
	50,000	100	0.658	**0.675**	**0.703**	0.653	0.623	0.627	0.615
	IAM	4.1	0.658	**0.731**	**0.769**	**0.698**	0.656	**0.666**	0.651
	SMM	12.4	0.658	**0.717**	**0.755**	**0.702**	**0.664**	**0.676**	**0.664**
Mismaloya ($n = 25$)	1000	2	0.667	**0.737**	**0.764**	**0.676**	0.624	0.626	0.604
	2000	4	0.667	**0.697**	**0.735**	0.653	0.604	0.607	0.591
	4000	8	0.667	**0.668**	**0.703**	0.632	0.584	0.593	0.577
	6000	12	0.667	0.649	**0.683**	0.613	0.571	0.577	0.563
	50,000	100	0.667	0.502	0.531	0.465	0.429	0.430	0.415
	IAM	2.2	0.667	**0.731**	**0.758**	**0.675**	0.624	0.623	0.603
	SMM	4.7	0.667	**0.691**	**0.725**	0.649	0.600	0.603	0.587
Playa Ticuiz ($n = 15$)	1000	2	0.591	**0.724**	**0.751**	**0.660**	**0.610**	**0.607**	0.584
	2000	4	0.591	**0.785**	**0.705**	**0.620**	0.570	**0.610**	0.553
	4000	8	0.591	**0.626**	**0.662**	0.581	0.532	0.537	0.520
	6000	12	0.591	**0.596**	**0.629**	0.555	0.509	0.513	0.496
	50,000	100	0.591	0.394	0.419	0.357	0.324	0.323	0.311
	IAM	3.6	0.591	**0.682**	**0.711**	**0.624**	0.576	0.577	0.557
	SMM	9.9	0.591	**0.608**	**0.642**	0.564	0.520	0.525	0.508
Boca de Apiza ($n = 21$)	1000	2	0.637	**0.734**	**0.761**	**0.674**	0.623	0.622	0.599
	2000	4	0.637	**0.694**	**0.729**	**0.646**	0.596	0.599	0.582
	4000	8	0.637	**0.657**	**0.695**	0.618	0.577	0.578	0.563
	6000	12	0.637	0.637	**0.675**	0.599	0.577	0.562	0.546
	50,000	100	0.637	0.476	0.582	0.440	0.403	0.404	0.457
	IAM	3.5	0.637	**0.700**	**0.734**	**0.651**	0.600	0.604	0.584
	SMM	9.7	0.637	0.646	**0.683**	0.608	0.566	0.573	0.557
Barra de la Cruz ($n = 24$)	1000	2	0.631	**0.731**	**0.760**	**0.760**	0.622	0.619	0.598
	2000	4	0.631	**0.695**	**0.726**	**0.640**	0.594	0.596	0.580
	4000	8	0.631	**0.655**	**0.691**	0.615	0.569	0.575	0.558
	6000	12	0.631	**0.634**	**0.668**	0.595	0.553	0.557	0.543
	50,000	100	0.631	0.466	0.495	0.498	0.394	0.394	0.381
	IAM	3.9	0.631	**0.693**	**0.726**	**0.643**	0.594	0.596	0.578
	SMM	11.4	0.631	**0.637**	**0.672**	0.597	0.554	0.559	0.545
Puerto Arista ($n = 28$)	1000	2	0.651	**0.739**	**0.766**	**0.679**	0.628	0.629	0.610
	2000	4	0.651	**0.704**	**0.741**	**0.659**	0.610	0.615	0.601
	4000	8	0.651	**0.673**	**0.712**	0.642	0.595	0.599	0.588
	6000	12	0.651	**0.659**	**0.695**	0.625	0.584	0.592	0.575
	50,000	100	0.651	0.524	0.552	0.489	0.450	0.453	0.440
	IAM	2.9	0.651	**0.721**	**0.751**	**0.667**	0.619	0.621	0.605
	SMM	7.1	0.651	**0.679**	**0.716**	0.644	0.596	0.606	0.589

(Ne) effective population size, $\Theta = 4\ N_e\mu$, (IAM) infinite alleles mutation model, (SMM) step-wise mutation model, P_g = proportion of multi-step mutations, δ_g = mean size of multi-step mutations. M_C (*M* critical) values higher than observed *M*-ratios are in bold.

such as reproductive variance (unequal reproductive output from females to a cohort) can also affect N_e, and therefore impact the estimation of *M*-ratios.

In Mediterranean loggerhead turtles, nuclear DNA analyses revealed no evidence of population bottlenecks, even when fisheries, egg harvest and tourism development had considerable effect on their populations in the last decades (Carreras et al., 2007). Carreras et al. (2007), suggested that other variables such as male-mediated gene flow and the existence of stepping stone colonies in the region might have contributed to the maintenance of allelic and genetic variability in this species. On the other hand, uncertainty regarding variation of mutation process, particularly for microsatellite markers, number of molecular markers used, and small sample sizes could have influenced the detection of

Table 4
Pairwise comparisons of F_{ST} (below the diagonal) and D_{EST} (above the diagonal) for 13 nesting areas of olive ridley turtles in Mexico. Bold indicate significant values ($P < 0.05$).

	BCP	EVE	PLA	NVA	PVG	MIS	PTI	BAP	TCO	SJC	BCR	ESC	PAR
BCP		0.05	0.02	0.05	0.05	**0.14**	0.06	0.02	0.03	**0.08**	0.04	0.03	**0.14**
EVE	0.01		0.04	0.10	0.07	0.04	**0.15**	**0.09**	0.08	**0.12**	0.09	0.05	**0.18**
PLA	0.009	0.005		0.07	0.04	**0.12**	0.06	0.05	0.06	**0.11**	0.07	0.05	**0.15**
NVA	0.007	0.02	0.01		0.09	**0.19**	0.04	0.05	0.04	**0.14**	0.07	0.02	**0.19**
PVG	0.01	0.02	0.004	0.01		**0.16**	0.04	−0.003	−0.03	0.07	0.02	0.04	0.06
MIS	**0.02**	0.01	0.02	**0.04**	**0.04**		**0.22**	**0.17**	**0.18**	**0.22**	**0.18**	**0.15**	**0.26**
PTI	0.002	0.02	0.0005	0.009	−0.0005	**0.04**		0.05	0.02	0.06	0.02	0.06	0.06
BAP	0.001	**0.02**	0.006	0.01	0.006	**0.03**	0.004		−0.03	**0.12**	0.01	0.04	0.08
TCO	0.005	0.01	0.01	0.008	−0.01	**0.04**	−0.0007	−0.01		**0.10**	0.002	0.02	0.06
SJC	**0.02**	**0.03**	**0.02**	**0.03**	**0.01**	**0.05**	0.008	**0.03**	**0.02**		0.009	**0.07**	**0.08**
BCR	−0.02	−0.007	−0.01	−0.004	−0.02	0.004	−0.01	−0.01	−0.03	−0.01		0.004	0.06
ESC	0.006	0.01	0.01	0.003	0.009	**0.03**	0.008	0.01	0.008	**0.01**	−0.02		**0.15**
PAR	**0.02**	**0.03**	**0.02**	**0.03**	0.01	**0.06**	0.008	0.01	0.0008	**0.02**	−0.02	**0.03**	

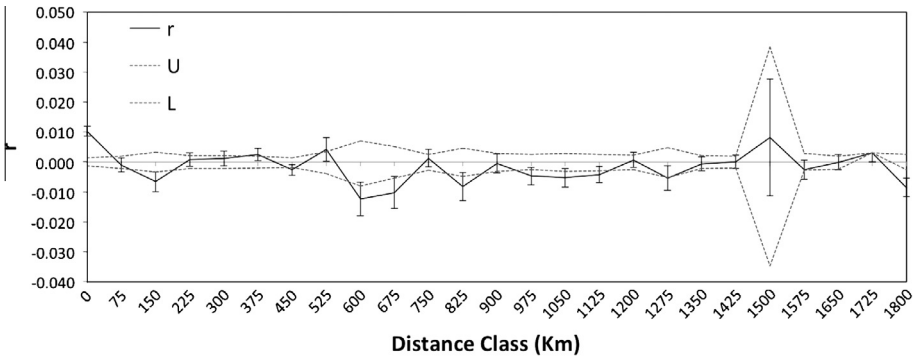

Fig. 2. Spatial autocorrelation coefficient (r) for nesting colonies of olive ridleys in Mexico over a range of distance classes. The permuted 95% confidence interval (dashed lines; upper (U) and lower (L) confidence limits) and the bootstrapped 95% confidence error bars are also shown.

bottlenecks. In our case, using a large sample ($n = 334$) and exploring models that encompass a reasonable range of variation for both P_g (0.10–0.57) and δ_g (3.1–4.0) and various values of theta, allowed us to confidently assess reductions of N_e. Given that specific parameters available for olive ridleys were derived from only two microsatellite markers, we favor the model proposed by Peery et al. (2012) as a more robust choice of parameters. Finally, M-ratio can be less sensitive to the reintroduction of rare alleles by high levels of gene flow in contrast to the heterozygosity excess method implemented in BOTTLENECK (Williamson-Natesan, 2005), which also has a reduced power of detection compared to M-ratio test (Garza and Williamson, 2001).

4.2. High connectivity along the Mexican coast

Our analyses disclosed high levels of connectivity among most nesting colonies and very low population substructure for olive ridleys in Mexico. Shallow genetic structure was expected considering the scenario of recent colonization of the Eastern Pacific by olive ridleys around 0.3 million years ago (Shanker et al., 2004), and the possible recent divergence of populations with large effective population sizes. Previous studies also suggested a lack of nuclear differentiation in olive ridleys (Bowen et al., 1998; Shanker et al., 2004). Our results did not support previous mitochondrial-based findings of a divergent population in Baja California (López-Castro and Rocha-Olivares, 2005). Genetic discordances between mtDNA and nDNA have been reported for other sea turtles due to strong matrilineal population structure and substantial male-mediated gene flow among nesting colonies (Bowen et al., 2005; Roberts et al., 2004). In addition, mark-recapture data for the species shows high levels of exchange indicating some degree of flexibility in nesting site fidelity, potentially associated with opportunistic behavior to explore new areas (Morreale et al., 2007). Finally, the lack of correlation observed between genetic differentiation and geographic distance (Fig. 2) is consistent with the long-distance dispersal pattern reported for olive ridleys in other regions (see Shanker et al., 2004).

Despite the overall finding of reduced population structure, our spatial autocorrelation analysis shows that individuals sampled in the same nesting beach have greater-than-random genetic similarity (Fig. 2), suggesting that females exhibit some degree of fidelity to nesting sites. In addition, the nesting colonies of Mismaloya, San Juan de Chacahua and Puerto Arista accounted for most of the low but statistically significant genetic differentiation (Table 4). It is possible that the demes at Mismaloya and Puerto Arista, which show a signal of bottlenecks, were also impacted at the level of allele frequency. Alternatively, one can speculate on environmental and topographic features impacting on these subpopulations. For instance, the oceanographic system of the Tehuantepec Gulf (Fiedler, 2002) may influence dispersal of individuals reaching the nesting colony of Puerto Arista during the breeding season. This and other competing hypotheses should be investigated by seascape genetics studies designed to statistically assess the interactions between environmental features and evolutionary processes, such as gene flow, in olive ridley nesting colonies.

4.3. Conservation implications for olive ridleys in Mexico

The olive ridley sea turtle was listed as 'endangered' in the IUCN Red List until 1996. Recent global population assessments have placed this taxon under the 'vulnerable' category, in which species are considered to have declined by 30% and 50% (Abreu-Grobois and Plotkin, 2008). This general recovery may reflect conservation policies and practices in recent decades. However, the limited and unevenly distributed data across oceanic regions and possible bias from well monitored (and therefore better protected) nesting

colonies is recognized as the main limitation of the current olive ridley population assessment.

Our results indicated low but biologically relevant population genetic structure in Mexico suggesting that a few colonies might behave as independent demographic units with differing population dynamics over time. In this context, barriers to dispersal (i.e. oceanographic currents) can reduce the probability of colonization on depleted areas and contribute to differences observed on trends of recovery among nesting sites (Briseño-Dueñas, 2007; Márquez-M et al., 2007). On the other hand, most of the colonies included in this study appear well connected over a vast spatial scale. The recovery of severely depleted colonies of sea turtles in Mexico (e.g. Escobilla) and elsewhere (e.g. Hawaiian green turtle; Balazs and Chaloupka, 2004; reviewed by Hays, 2004), give us an indication of the positive effects of long-term conservation efforts and expected timeframes for recovery.

Despite that conservation efforts on nesting beaches in Mexico have notably expanded in the last decade, the protection of olive ridleys in the sea continue to be challenging and important threats such as bycatch are still present. Moreover, changing environmental conditions could also be of particular relevance since sea turtles are particularly vulnerable to global issues such as climate change (Hays et al., 2003). A better understanding of the multiple interacting forces in driving recovery is needed to improve conservation actions and polices, especially when applied to marine ecosystems. To achieve recovery, marine species have almost entirely depended on the reduction of human impacts, particularly exploitation, habitat loss and pollution. However, the understanding of demographic changes at a deme level is highly relevant to accurately address the cumulative impact of human activities; such as magnitude of depletion, allele effects, genetic diversity, population and metapopulation structure (Lotze et al., 2011). Thus, an enhanced understanding of each of these factors and their interaction with major drivers is needed to allow for improved conservation of sea turtles.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biocon.2013.09.009.

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