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ABSTRACT

Humpback whales (*Megaptera novaeangliae*) congregate in winter in the Mexican Pacific and Central America coastal waters. According to NOAA, based on the results of the SPLASH project, these congregations belong to two Different Population Segments (DPS): the DPS Central America, in danger, and the DPS Mexico, threatened. This study did not consider in this division the Humpback whales distributed in the southeastern Mexican Pacific from Colima to Oaxaca. The present study was focusing on analyzing the relationship of the humpback whales from southern Mexico with whales from other regions in the Mexican Pacific and Central America. The photo-id matching included 7,250 individuals from 6 region's catalogs of the Mexican Pacific (BCS; Sinaloa; Nayarit-Jalisco; Colima; Guerrero and Oaxaca). The highest Recapture Index was among the whales from Colima, Guerrero, and Oaxaca in southern Mexico. The haplotypic frequency of 51 skin samples collected in Oaxaca (48) and Guerrero (3) showed significant differences with the other three breeding sites studied in Mexico (Baja California, Bahía de Banderas, and Revillagigedo Archipelago). In contrast, there were no significant differences with the humpback whales from Central America. The photo-identification results indicate that the whales from Colima to Oaxaca belong to the same congregation, and the genetics show that these whales form the same population unit with the whales of Central America.

Key Words: Humpback Whale, Photo-identification, Haplotypes, Population Unit

INTRODUCTION

The coasts of Mexico and Central America are breeding grounds of the Humpback whales (*Megaptera novaeangliae*) in the North Pacific (Rice, 1978). From 2004 to 2006 the SPLASH project studied the abundance, movements, and migratory destinations of humpback whales (Calambokidis *et al.*, 2008; Barlow *et al.*, 2011; Baker *et al.*, 2013). As a result of this project, two population units in the Mexican Pacific were identified: the "offshore," including the Revillagigedo Islands and the "coastal" in the coasts of Jalisco, Nayarit, and Sinaloa. Both population units overlap in the waters of the Baja California Peninsula and the mainland (Gonzalez-Peral, 2011; Urbán *et al.*, 2017). The fieldwork in Mexico during this project was in the northern Pacific (Nayarit/Jalisco and Baja California Peninsula) and the offshore islands of Revillagigedo. No effort was made in southern Mexico.

According to NOAA, there are two Different Population Segments (DPS) in the region. The DPS “Central America,” in danger, and the DPS “Mexico” threatened. This study aims to analyze the population assignment of the humpback whales from southern Mexico with whales from other population units in the Mexican Pacific and Central America.

METHODS

We use the photo-identification to estimate the movements of humpback whales from southern Mexico with other regions in the Mexican Pacific. We compare a total of 7,250 identified individuals from 6 regional catalogs: Baja California Sur (2455); Sinaloa (690); Nayarit-Jalisco (2780); Colima (47); Guerrero (332) and Oaxaca (254)] (Fig.1).

The matching was by eye and with the support of the HotSpotter program. Subsequently, we estimate the Recapture Interchange Index (RII) (Baker *et al.*, 1985; Calambokidis *et al.*, 2000; Urban *et al.*, 2000), to know the degree of exchange among the regions.

In relation with the differences of the haplotypic frequencies of the humpback whales from southern Mexico, Central America and Baja California Peninsula, we collected 51 skin samples during the winter seasons of 2018 and 2019 from southern Mexico: Oaxaca (48) and Guerrero (3); and from Baja California Peninsula (126) (Fig.1) We analyzed 500 base pairs from the Control Region of Mitochondrial DNA, and amplified with the help of the Polymerase Chain Reaction (PCR) technique to obtain the sequences, identify the variable sites, and describe the haplotype frequencies present in this congregation. Then we use the F_{ST} statistical test to find out the significant differences (p-value) between populations. Finally, the results of these frequencies were compared with those previously described by González-Peral (2011) and Baker *et al.*, (2013), from the SPLASH project, from Bahía de Banderas; Revillagigedo Islands; Baja California Peninsula; and Central America.

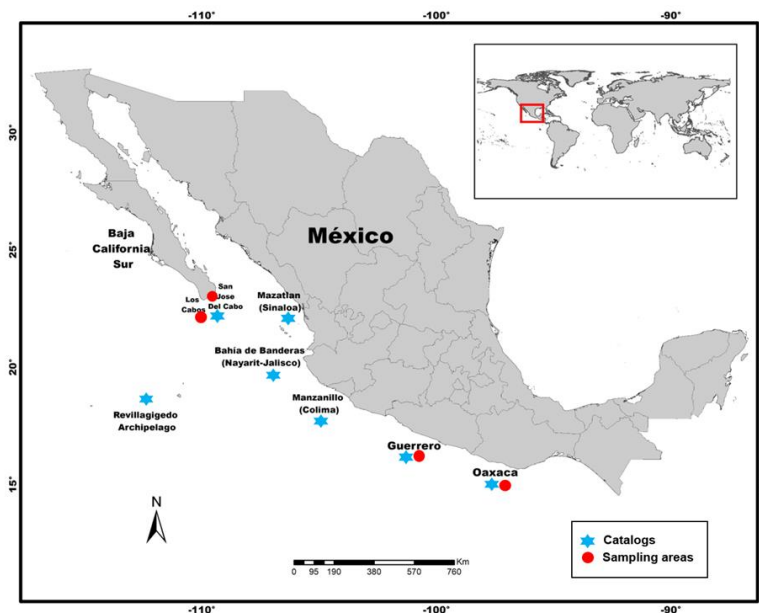


Figure 1. Mexican Pacific study areas.

RESULTS

Photo-Id

The higher RII values were between, Oaxaca-Guerrero (0.83), and Guerrero-Colima (0.45), following by Colima-Nayarit/Jalisco (0.20), Colima-Oaxaca (0.17), and Guerrero-Nayarit/Jalisco (0.17). The lower RII was between Revillagigedo and all the other regions (Table 1). There is a gradual diminution of the RII among the coastal regions from south to north.

Table 1. Interchange Index. Higher values orange and green, lower values pink.

	OAX (254)	GRO (332)	COL (47)	NY/JAL (2780)	BCS (2455)	SIN (690)	REV (692)
OAX	•	70	2	108	65	4	2
GRO	*0.8300	•	7	154	94	17	0
COL	0.1675	*0.4486	•	26	12	3	0
NAY/JAL	0.1529	*0.1668	*0.1989	•	339	152	37
BCS	0.1042	0.1165	0.1039	0.0496	•	98	28
SIN	0.0228	0.0742	0.0925	0.0792	0.0578	•	5
REV	0.0113	0	0	0.0192	0.0164	0.0104	•

Haplotypic Frequencies

The 126 humpback whales samples from Baja California Peninsula presented 17 different haplotypes where A- and A + represent the 34% of the samples, and E1 and F2 the 35%; in contrast the 51 humpback whale samples from southern Mexico where different with 9 different haplotypes where E1 and F2 represent the 76% (Fig.2, Table 2).

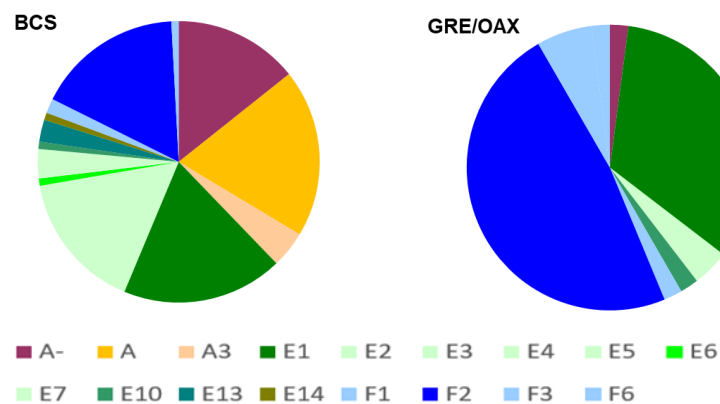


Figure 2. Haplotype frequencies from Baja California Sur (BCS) and Guerrero/Oaxaca (GRE/OAX)

Table 2. Percentage of the different haplotypes present in the humpback whales' samples from BCS (126) and GRO/OAX (51).

	A-	A+	A3	E1	E2	E3	E4	E5	E6	E7	E10	E13	E14	F1	F2	F3	F6
GRO/OAX	2	0	0	34	0	0	2	2	0	0	2	0	0	2	48	6	2
BCS	14	19	4	19	0.8	5	6	4	0.8	3	0.8	3	0.8	2	17	0	0.8

To find if the haplotype frequencies changed from 2004-2006 (323 samples), during the SPLASH project (from González-Peral, 2011), to 2018-2019 (126 samples), we compare samples from Baja California Sur, and there were no significant differences (Fig. 3).

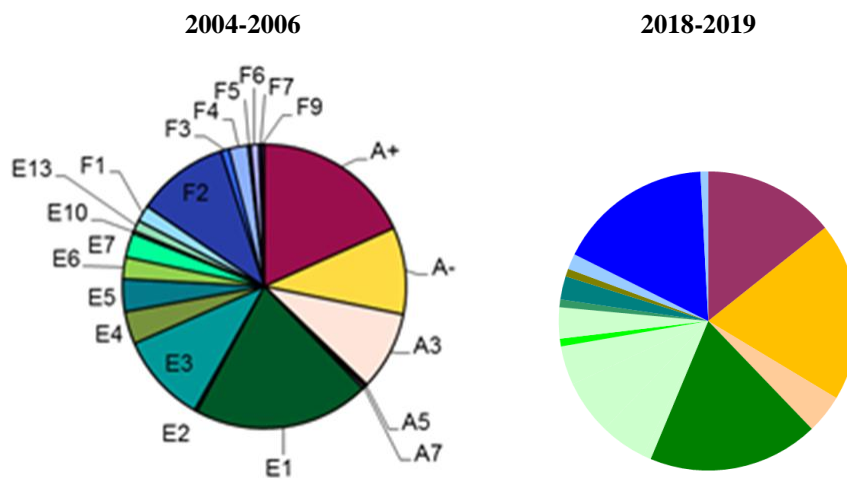


Figure 3. Haplotype frequencies from Baja California Sur 2004-2006 and 2018-2019.

Finally, we compare the haplotype frequencies from Guerrero-Oaxaca in southern Mexico with the haplotype frequencies analyzed in the SPLASH project from three regions in Mexico (Revillagigedo Islands, Baja California Peninsula and Nayarit/Jalisco in the mainland) (from González-Peral, 2011), and Central America (from Baker *et al.*, 2013). All comparisons were different, except Guerrero/Oaxaca with Central America (Table 3, Figure 4).

Table 3. Φ FST values and significant differences in the comparison of haplotype frequencies of humpback whales from Revillagigedo (REV), Baja California Sur (BCS, Nayarit/Jalisco (NAY/JAL), Guerrero/Oaxaca (GRO/OAX) and Central America. The number of samples in parentheses. Sign (-) and yellow = no significant difference ($p > 0.05$).

Φ FST/Significance P-value	REV (395)	BCS (449)	JAL/NAY (317)	GRO/OAX (51)	CAM (43)
REV		+	+	+	+
BCS	0.03327		+	+	+
NAY/JAL	0.08273	0.00953		+	+
GRO/OAX	0.47185	0.26214	0.17012		-
CAM	0.33562	0.13479	0.06166	0.01139	

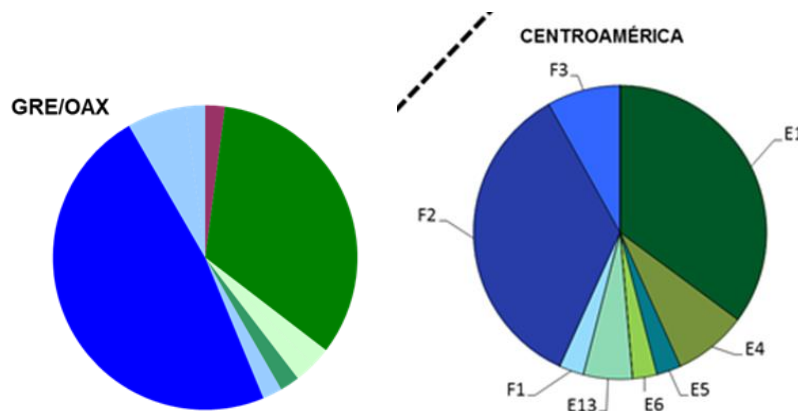


Figure 4. Haplotype frequencies from GRO/OAX in southern Mexico and Central America.

DISCUSSION

The results of the photo-identification matching show movement of the whales along with the coast of the Mexican Pacific, with a strong affinity in the southern regions, Oaxaca, Guerrero, and Colima with higher RII. Nevertheless, there is a not clear division between the northern coastal regions of NAY/JAL, SIN, or the Baja California Peninsula with the southern regions. A possible explanation is that the matches between southern Mexico with the northern regions represent whales in transit from southern Mexico to or from the feeding areas on the west coast of the USA. Humpback whales from Central America migrate to California, Oregon, and Washington to feed during the summer, based on photo-identified whales (Calambokidis *et al.*, 2000; 2008), and more recently, base on mtDNA analysis (Lohman *et al.*, 2020; Martien *et al.*, 2020).

The only knowledge on the migratory route from the west coast of the USA to Central America is from a whale tagged in central California in 2005 that migrated along the coast to Guatemala (Mate *et al.* 2018). Additionally, another whale tagged in southern Oregon in the same year was also

tracked migrating to a breeding destination before its tag stopped transmitting off Michoacán, Mexico (Mate *et al.* 2018). For the latter animal, we do not know whether it stayed in the Mexican breeding area or if it continued traveling further south to Central America. In both cases, the animals travel close to the west coast of Baja California Peninsula until the southern tip at Los Cabos and then cross the mouth of the Gulf of California until the coast of Nayarit and continue to the southeast, one until Michoacán, between Colima and Guerrero, and the other continued until Guatemala, in Central America.

In contrast, haplotype frequencies show a clear genetic difference from southern Mexico with the rest of the Mexican Pacific and a strong affinity with the humpback whales from Central America. A possible explanation about the differences in the haplotype frequency between the northern and southern whales from the coast of Mexico because the Baja California Peninsula, Sinaloa and Nayarit/Jalisco is an overlapping zone of humpback whales from the "offshore" population unit with predominant haplotypes -A and +A, and whales from the "Costal" population with the predomination of the haplotype F2 (González-Peral, 2011; Urbán *et al.*, 2017).

In summary, the humpback whales from southern Mexico belong to the Central American population, and there is not a clear boundary between the northern and southern coastal humpback whales in the Mexican Pacific. Future steps include the comparison of the photo-id catalogs from the Central America humpback whales and Mexico to know the movement of the whales in the region and develop an abundance estimation.

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