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INTERNATIONAL
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ABSTRACT

Humpback whales (*Megaptera novaeangliae*) congregate in winter in the Mexican Pacific and Central America for their breeding and breeding activities. 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. Humpback whales distributed in the southeastern Mexican Pacific from Colima to Oaxaca were not considered in this division. This study focusing on analyze the relationship of the humpback whales from southern Mexico with the whales from other regions in the Mexican Pacific and Central America. Photo-identification, techniques and analysis of their haplotypic frequencies were used. To know their movements, 7,250 individuals from 6 regions of the Mexican Pacific (BCS; Sinaloa; Nayarit-Jalisco; Colima; Guerrero and Oaxaca) were compared. The highest Recapture Index was among the whales in Colima, Guerrero and Oaxaca. At the same time, the haplotypic frequency of 51 skin samples collected in Oaxaca (48) and Guerrero (3) was determined, which showed significant differences with the three breeding sites studied in Mexico (Baja California, Bahía de Banderas and Revillagigedo Archipelago); and in contrast, no significant differences were observed with 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 important breeding grounds of the Humpback whales (*Megaptera novaeangliae*) in the North Pacific (Rice, 1978). In the SPLASH project the abundance, movements and migratory destinations of humpback whales were determined (Calambokidis *et al*., 2008; Barlow *et al*., 2011; Baker *et al*., 2013). As a result of this project, 2 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 Baja California Peninsula and mainland (Gonzalez-Peral, 2011; Urbán *et al*., 2017). The field work in Mexico during this project was dedicated to the northern Pacific (Nayarit/Jalisco and Baja California Peninsula) and the offshore islands of Revillagigedo. No effort was dedicated to 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. The aim of this study is analyze the relationship of the humpback whales from southern Mexico with the whales from other population units in the Mexican Pacific and Central America.

METHODS

To know the movements of humpback whales from southern Mexico in relation with other regions in the Mexican Pacific, we use the photo-identification technique. We compare a total of 6,558 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, the Recapture Interchange Index (RII) was calculated (Baker *et al*., 1985; Calambokidis *et al*., 2000; Urban *et al.*, 2000), to know the degree of exchange among the regions.

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

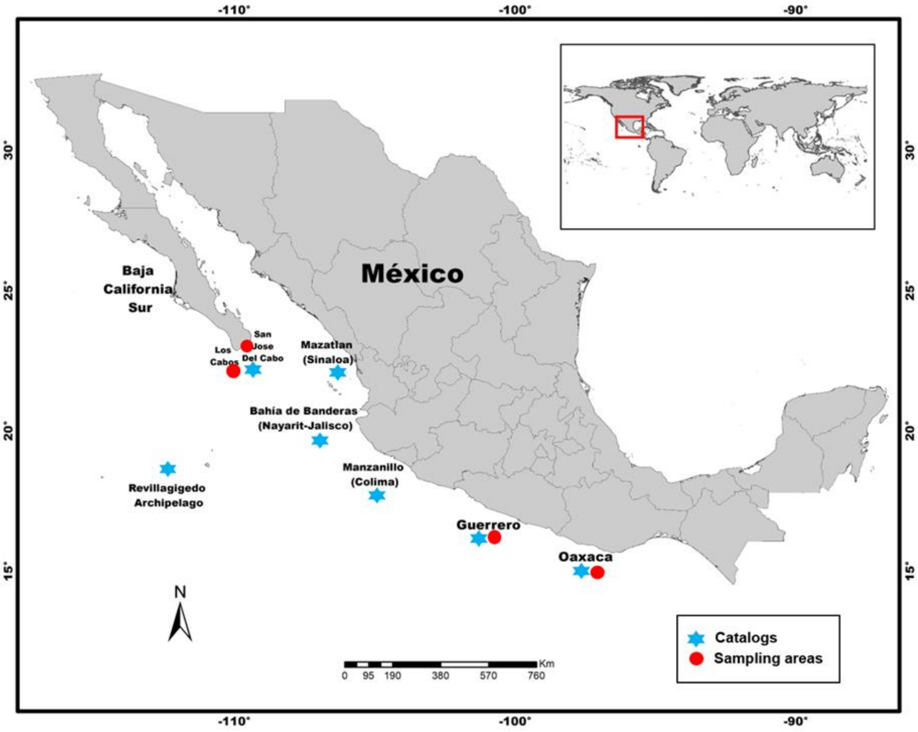


Figure 1. Mexican Pacific study areas.

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 were 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 and (▫) lower values.

	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 significatively different with 9 different haplotypes where E1 and F2 represent the 76% (Fig.2, Table 2).

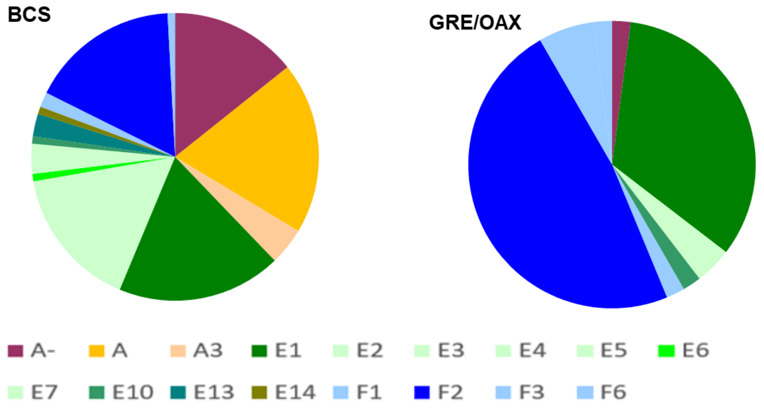
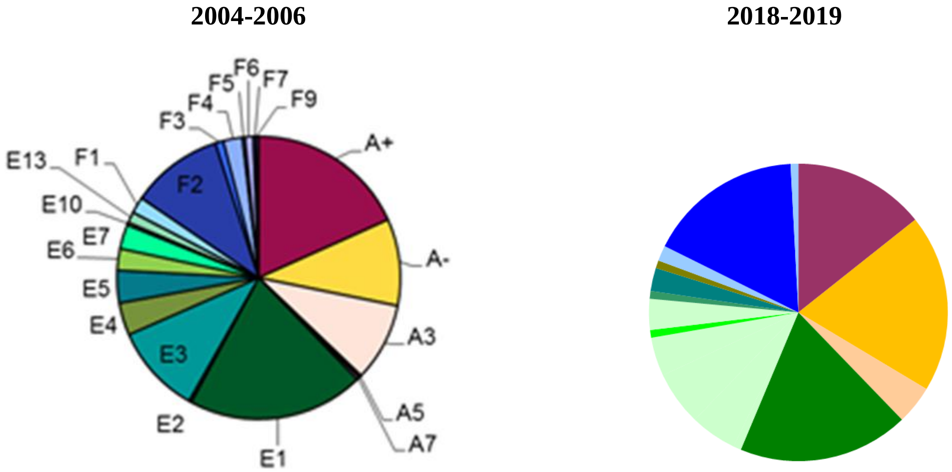


Figure 2. Haplotype frequencies from Baja California Sur (BCS) and Guerrero/Oaxaca (GRE/OAX)

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 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).



2004-2006

2018-2019

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 mainland) (from González-Peral, 2011), and Central America (from Baker *et al.*, 2013). All comparisons were significatively different except Guerrero/Oaxaca with Central America (Table 3, Figure 4).

Tabla 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. Number of samples in parentheses. Sign (-) and yellow = no significant difference (p> 0.05).

ΦFST/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	

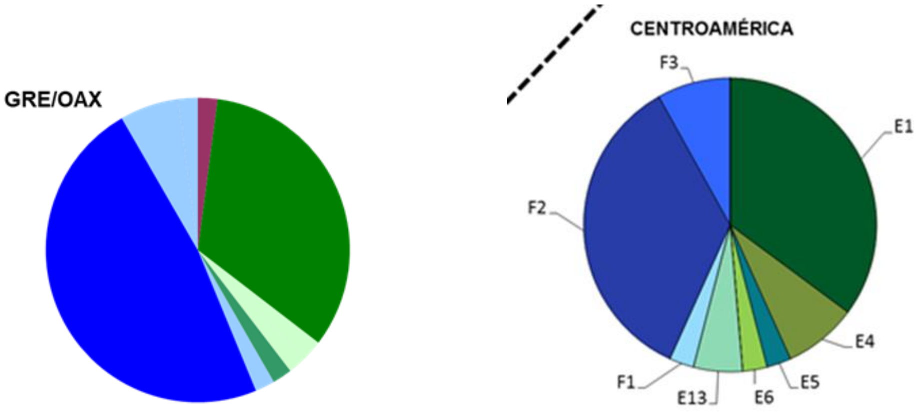


Figure 4. Haplotype frequencies from GRO/OAX in southern Mexico and Central America.

DISCUSSION

The results of the photo-identification matching catalogs show movement of the whales along the cost 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 coast regions of NAY/JAL, SIN, or the Baja California Peninsula with the southern regions. A possible explanation is that the matches between the southern Mexico with the northern regions represent whales in transit from southern Mexico to or from the feeding areas in the west coast of USA (Calambokidis et al., 2000; 2008).

In contrast, haplotype frequencies show a clear difference with the southern Mexico with the rest of the Mexican Pacific and a strong affinity with the humpback whales from Central America. A possible explanation between the photographic and genetic relations among the Mexican regions could be that the northern coastal regions and Baja California Peninsula is an overlapping zone of humpback whales from the "offshore" population unit with a predominant haplotypes -A and +A that influence the haplotype frequency of the whales in the region (González-Peral, 2011; Urbán *et al*., 2017).

In summary, the humpback whales from southern Mexico belong to the Central America 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 abundance estimation.

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