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Short Note

A Mother–Calf Humpback Whale (*Megaptera novaeangliae*) Pair from the Southeast Pacific Population Sighted in Mexican Waters

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The humpback whale (*Megaptera novaeangliae*) is a species with cosmopolitan distribution (Clapham & Mead, 1999); within the large population of the North Pacific Ocean, five Distinct Population Segments (DPSs) have been recognized (Bettridge et al., 2015). Individuals belonging to at least two DPSs (Mexican and Central American) feed from Alaska to California in June through October and then migrate to the coastal waters of Mexico and Central America in October through May to breed (Acevedo & Smultea, 1995; Urbán et al., 2000; Calambokidis et al., 2001, 2008; Alava et al., 2012; Bettridge et al., 2015; Figure 1). Meanwhile, humpback whales from the Southeast Pacific (SEP) DPS feed in waters off southern Chile and Patagonia and in waters surrounding the Antarctic Peninsula; they arrive at breeding grounds between May and December in coastal waters from Peru to Costa Rica (International Whaling Commission [IWC], 1998; Scheidat et al., 2000; Alava et al., 2012; Bettridge et al., 2015; Acevedo et al., 2017; Avila et al., 2020; Figure 1). Therefore, Central American coastal waters represent the potential range limit for different humpback populations/DPSs and correspond to an overlapping region for humpback whales distributed in the Northeast Pacific (NEP) and SEP (Acevedo & Smultea, 1995; Flórez-González et al., 1998; Calambokidis et al., 2001, 2008; Bettridge et al., 2015; Guzmán et al., 2015; Acevedo et al., 2017; Figure 1). However, individual whales can disperse beyond these "recognized" wintering ranges—for example, at least 33 humpback whale sightings were recorded between 2015 and 2018 in Nicaraguan waters belonging to the SEP, including some individuals observed in Antarctic feeding grounds (De Weerdt et al., 2020).

Calves usually have been observed during the period from November to May with a higher occurrence of newborns in February and March in the NEP, particularly off the mainland coast of Mexico; whereas calves have been observed from June to October, with a higher occurrence of newborn whales in August and September in the SEP (Chittleborough, 1958), particularly off Nicaragua (Medrano-González et al., 2010; De Weerdt et al., 2020).

This report describes an opportunistic sighting of a mother–calf humpback whale pair within the bays of Manzanillo, Colima, Mexico, on 21 September 2010 (19.0853°N, 104.3785°W; Figure 1), outside the usual period of calf registration in the NEP. The whales were sighted in the middle of the day mainly by fishermen in the region who alerted our research team. When we arrived at the site, the animals were near (~900 m) the rocky point of Santiago Bay, exhibiting a resting/nursing behavior. The two individuals remained together, executed a couple of breathing cycles, and then moved away from the bay. The duration of the sighting was approximately 1

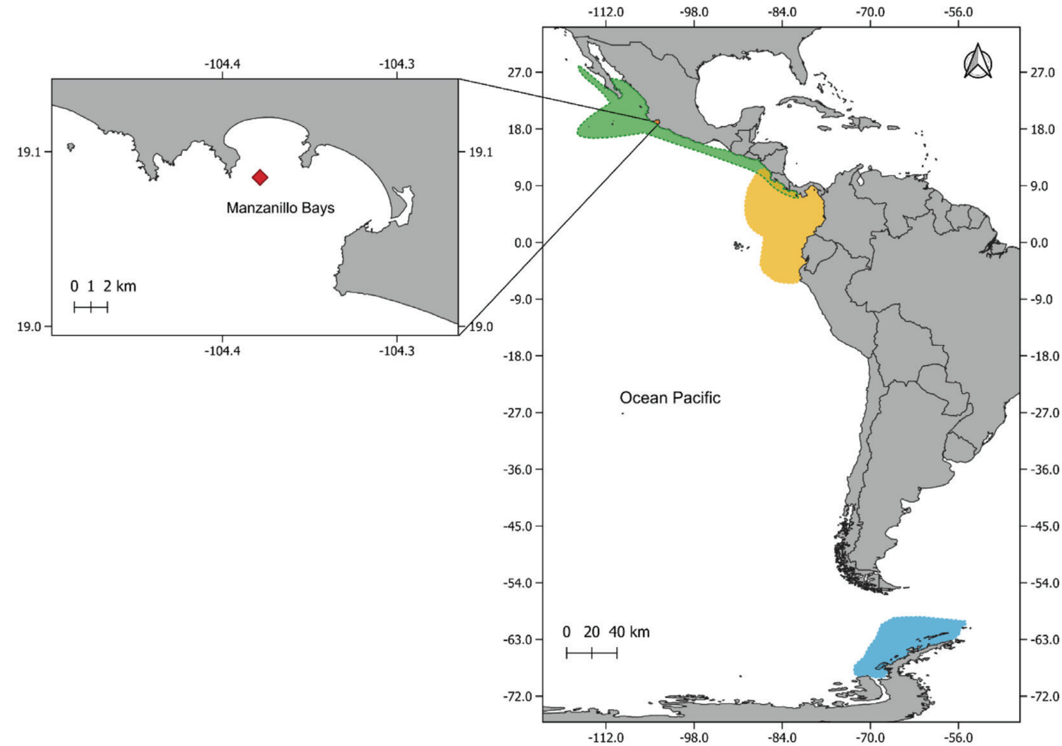

Figure 1. Location of the sighting point of the mother–calf pair in the bays of Manzanillo, Colima, Mexico, during September 2010 (red dot). Breeding grounds of humpback whales (*Megaptera novaeangliae*) are displayed both from the Northeast Pacific population in Mexico and Central America (green area) and the population of the Southeast Pacific in Central and South America (yellow area), as well as the potential feeding ground of this whale in the Antarctic Peninsula (blue area).

h, and no other humpback whales or other cetacean species were observed in the surrounding area. Mother and calf were not observed again in the following days.

During the sighting, photographs were taken of the dorsal fin of both individuals (Figure 2a & b) with a Canon EOS 60D digital camera with a 300 mm zoom lens because the mother's flukes were not shown in any of the breath-blowing cycles. Photographs of the calf were analyzed to estimate its relative age and total length. Based on Cartwright & Sullivan (2009), the calf's dorsal fin angle being > 72° and unfurled (Figure 2c) suggests that the calf's size was > 1/3 of the mother's size. This matches our estimate of the calf's total length being 5 to 6 m using our research vessel (~8 m) as reference. This size also coincided with the classification for calves (< 7 m) by Groch et al. (2018); thus, we inferred its age to be between 2 to 3 mo, with its birth likely occurring in June or July 2010. Hence, this calf's birth is consistent with the seasonal range for humpback calf sightings from the SEP DPS.

To identify the most plausible ocean basin origin of this humpback whale and its calf, comparisons of photo identifications, an analysis of mitochondrial (mt) DNA lineages, and assessment of stable isotope ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) were conducted.

Comparisons of Photo Identifications

Photographs of the mother's dorsal fin (Figure 2a) were compared to photo-identification catalogs of humpback whale dorsal fins from two breeding sites in Mexico (Southern Baja California: 166 individuals photographed in 1992 and 2004 to 2008; mainland coast of Bahía de Banderas: 270 individuals photographed in 1990 and 2006 to 2011), as well as a breeding site in Ecuador (59 mother–calf pairs photographed in 2010; courtesy of Fernando Félix). No matches were found between the whales photographed in Colima in September 2010, nor with the three catalogs of dorsal fins available. This result encourages further efforts to create photo-identification catalogs using the dorsal fin of individuals.

Figure 2. Humpback whale mother–calf pair sighted in September 2010 in the bays of Manzanillo, Colima, Mexico: (a) left side of the mother's dorsal fin, (b) left side of the calf's dorsal fin, and (c) back view of the calf's dorsal fin, showing a > 72°angle. (Photos provided by Christian D. Ortega Ortiz, Facultad de Ciencias Marinas, Universidad de Colima)

Analysis of Mitochondrial (mt) DNA Lineages

A skin biopsy was obtained from the mother using a 70-kg draw crossbow (Barnett Panzer V; Barnett Crossbows, Tarpon Springs, FL, USA) and a dart modified with a floating foam stopper into which a 15-mm long by 3-mm diameter stainless-steel tip was screwed; this semi-invasive procedure does not cause significant injury or disturbance to large cetaceans (Noren & Mocklin, 2012). The skin biopsy was subject to molecular (mitochondrial DNA) and chemical (stable isotope ratios) analyses. Given the maternal inheritance of mtDNA and the maternal transmission of migratory habits in humpback whales, the analysis of mtDNA variation in this species has been greatly informative regarding population identity, structure, and history (Baker et al., 1990, 1993).

To extract genomic DNA, 0.3 g of the epidermal layer from the biopsy were used and a 493-bp segment of the mtDNA control region was amplified by polymerase chain reaction to ultimately get the sequence of the amplified product with the Sanger method; the procedures employed were those of Baker et al. (2013). We compared the sequence of the mother humpback (hereafter referred to as "CS-2010") with mtDNA sequences from the NEP (González Peral, 2011; Baker et al., 2013) and SEP DPSs (Olavarría et al., 2007). Sequences were aligned by ClustalW with the software *MEGA*, Version 10.2.2, and trimmed to a 466-bp length using the sequence size of the haplotypes recorded in the Southeast Pacific as reference (Olavarría et al., 2007). Haplotype identity was then evaluated with a nucleotide ratio analysis among determined haplotypes for the mother and previously recorded haplotypes for the NEP and SEP using a Bayesian inference method with the model HKY + G (utilizing *MEGA* software). Analyses were carried out with a Markov Chain Monte Carlo with 25,000,000 generations and a sampling frequency of 1,000 using the software *Mr. Bayes*, Version 3.2.6.

The haplotype identified for the humpback whale mother was E13/SP8, belonging to the AE clade (Olavarría et al., 2007; Baker et al., 2013). However, this haplotype occurs in both the North and South Pacific populations. In the NEP, it is known as "E13" and has been reported in feeding areas ranging from British Columbia to California–Oregon, while in breeding areas, it has been detected in Mexico and Central America

(Baker et al., 2013). In the SEP, it is named "SP8" and has been recorded only in the breeding area of Ecuador–Colombia, with the Antarctic Peninsula as feeding grounds (Olavarría et al., 2007; Felix et al., 2012). The originating population of this humpback whale cannot be elucidated from its mtDNA lineage because the lineage is shared between the North and South Pacific populations due to gene flow.

Assessment of Stable Isotope Ratios of Carbon ($\delta^{13}C$) and Nitrogen ($\delta^{15}N$)

A skin biopsy was analyzed using this technique. The sample was preserved in a cryogenic vial in a container with liquid nitrogen. It was washed in the laboratory with distilled water and dried for 24 h in a lyophilizer. Lipids were extracted using a 1:1 chloroform/methanol, and the moisture-free sample was ground using an agate mortar. A subsample of 0.8 to 1.2 mg was weighed and sent to be processed in a mass spectrometer to obtain $\delta^{13}C$ and $\delta^{15}N$ values. These stable isotopes provide information about trophic relationships among organisms but also about their feeding migratory destinations (Witteveen et al., 2009a). Such ratios are expressed as enrichment values of the heavier isotope with reference to known standards (e.g., Todd et al., 1997; Witteveen et al., 2012).

Isotopic values obtained for CS-2010 were -24.98‰ for $\delta^{13}C$ and 7.23‰ for $\delta^{15}N$. These ratios showed some correspondence with the isotopic signal of South Pacific whales that feed off the western coast of the Antarctic Peninsula (Seyboth et al., 2018) and in Area V of the eastern Antarctic (Eggebo et al., 2022) (Table 1). A comparison with isotopic values reported for North Pacific humpback whales showed no such correspondence. As the value of one isotope (e.g., $\delta^{15}N$) approached values found in North Pacific whales, the other isotope (e.g., $\delta^{13}C$) was greater than the values found in North Pacific whales and vice versa (e.g., western Gulf of Alaska and Bering Sea; Table 1). Similarly, Antarctic minke whales (*Balaenoptera bonaerensis*) and fin whales (*Balaenoptera physalus*) that fed in waters off the Antarctic Peninsula also showed $\delta^{13}C$ values ranging from -25.6 to -23.1‰ and $\delta^{15}N$ values ranging from 7.8 to 8.7‰ (Seyboth et al., 2018).

Stable isotopes can be used as a regional approach for consumers due to the tendency for an inverse relationship between latitude and baseline isotopic values that is typically observed between mid and high latitudes, which are related to increased solubility of CO_2 (^{13}C-depleted) at low seawater temperatures (Goericke & Fry, 1994; France, 1995) and to decreased intensity of bacterial denitrification from mid to high latitudes, giving as a result decreased baseline $\delta^{15}N$ values towards high latitudes (Altabet et al., 1999; Voss et al., 2001). These low isotope values at the baseline are reflected in top predators such as marine mammals (Burton & Koch, 1999; Witteveen et al., 2009b; Elorriaga-Verplancken et al., 2018).

Therefore, our complementary results—that is, the date when the humpback whale pair was recorded—and C and N isotopic values pointed to the SEP DPS as the origin of CS-2010. Thus, this whale may represent the first and so far only documented record of a humpback whale from the SEP DPS nursing in the NEP; it exceeded the usual limits of distribution between June and October (i.e., in coastal waters from Peru to Costa Rica) to the Mexican coast to carry out activities associated with reproduction such as nursing. This whale probably fed in Antarctic waters due to the coincidence of its isotopic values with those previously reported in whales feeding in that region, which would suggest that this will be one of the longest migrations for this species (~10,800 to 12,000 km; Figure 1) when considering the three

Table 1. $\delta^{13}C$ and $\delta^{15}N$ values of humpback whale (*Megaptera novaeangliae*) individuals sampled in feeding areas from the Northern and Southern Pacific. Extreme high or low isotope values by isotope ratio and region are marked in bold.

Regions	Areas	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
Northern Pacific	Western Gulf of Alaska	**-23.0**, -15.8	11.3, 15.3
	California and Oregon	-17.9, **-15.2**	11.8, **16.6**
	Bering Sea	-19.6, -15.9	**7.4**, 15.7
Southern Pacific	Gerlache, Antarctic	-25.3, -23.6	8.5, 8.7
	Bransfield, Antarctic	-23.1, **-21.6**	8.2, **11.6**
	Area V, Antarctic	**-27.35**, -23.52	**5.21**, 8.22
This study	Colima, Mexico	-24.98	7.23

Data taken from Witteveen et al. (2009b), Seyboth et al. (2018), and Eggebo et al. (2022).

possible migratory routes from the Antarctic Peninsula (i.e., the offshore, the great circle, and the coastal) (De Weerdt et al., 2020). This distance would exceed the largest reported migration of 10,005 km from Antarctica to Nicaragua (De Weerdt et al., 2020) and the largest movement between breeding areas of 9,800 km from Brazil to Madagascar (Stevick et al., 2011).

The environmental factor that mainly affects the ecology of marine mammals in the Eastern Tropical Pacific (ETP) is El Niño Southern Oscillation (ENSO). Effects of ENSO on feeding, physiology, and dispersion of marine mammals can be projected through changes in their population dynamics (e.g., Aurioles & LeBoeuf, 1991; Gardner & Cháves-Rosales, 2000; Urbán et al., 2003; Soto et al., 2004; Comisión Permanente del Pacífico Sur [CPPS] Programa de las Naciones Unidas para el Medio Ambiente [PNUMA], 2012; Elorriaga-Verplancken et al., 2016a, 2016b; Páez-Rosas et al., 2017). We compiled the monthly Oceanic Niño Index (ONI) values from the National Oceanic and Atmospheric Administration (NOAA) (Zhang et al., 1997; www.cpc.noaa.gov/products/analysis_monitoring/ensostuff/ensoyears.shtml) for 2006 to 2016 and examined its variation between June and December (3 mo before and after September, encompassing the humpback whale austral breeding season). The mean ONI for June to December was 0.02 ± 0.83 (standard deviation [SD]) for the entire 2006-2016 period. The mean ONI for June to December 2010 was -1.10 ± SD = 0.37. Year 2010 was unique during the examined period because of the steep decrease in ONI from January to September linked to the sudden change from the moderate El Niño event in 2009-2010 to the strong La Niña event in 2010-2011 (Kono-Martínez et al., 2017; Figure 3). In addition, the occurrence of thermal environmental variability causes cold surface waters to extend northward from the Equator so that the most suitable temperatures for the reproductive activities of this species occur further north of its wintering grounds (Pelayo-González et al., 2022).

CS-2010 represents the northernmost record of a humpback whale from the SEP, driven by oceanographic variability that occurred in the ETP. It is noticeable that the observed environmental conditions (i.e., cold waters in the ETP) favored the extralimital dispersion of CS-2010 and coincided with those inferred for historical gene flow by Medrano-González et al. (2001). Anomalous cold conditions that could expand typical foraging areas have also been related to extraordinary records of other southern species such as the

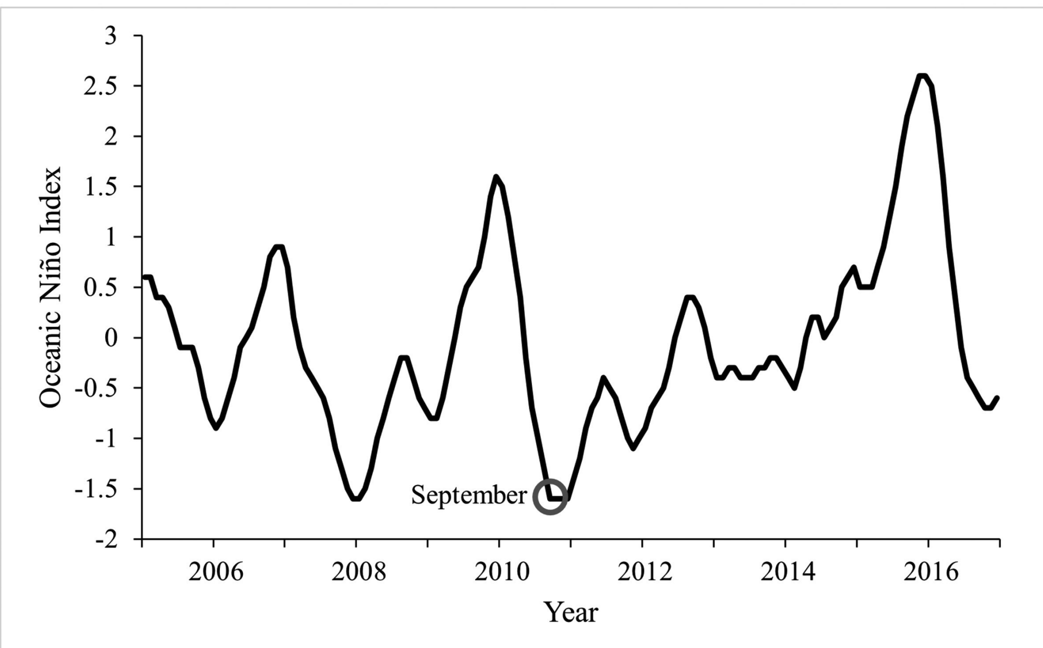

Figure 3. Variation of the Oceanic Niño Index between 2006 and 2016. The black circle indicates the record of the humpback whale mother in Colima, Mexico, in September 2010. Values compiled from NOAA (Zhang et al., 1997; www.cpc.noaa.gov/products/analysis_monitoring/ensostuff/ensoyears.shtml).

southern elephant seal (*Mirounga leonina*), which has been documented in the Northern Hemisphere in recent decades (Alava et al., 2022).

The ETP exhibits a unique oceanography and rich marine biodiversity supported by high productivity. It is also a region of high environmental variation because of ENSO (Ballance et al., 2006; Wang & Fiedler, 2006) and other phenomena such as the North Pacific heatwave called "The Blob" (Kintisch, 2015). The effects of such environmental changes on the migration and dispersion of humpback whales and other marine mammals, as suggested by CS-2010, might entail continuous change in the population structure of these animals that could be relevant for marine conservation over the long term—especially in the presence of an increased frequency of oceanographic anomalies in the Central Pacific in recent decades (Freund et al., 2019). Therefore, it will be important to maintain attention on more potential cases regarding trans-oceanic humpback whale migrations in the future.

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