LAJAM 5(2): 125-128, December 2006
e-ISSN 2236-1057 - doi:10.5597/lajam00104
http://dx.doi.org/10.5597/lajam00104
ISSN 1676-7497

GENETIC SEX DETERMINATION SUPPORTS THE GULF OF CALIFORNIA AS AN IMPORTANT HABITAT FOR MALE AND FEMALE SPERM WHALES (*PHYSETER MACROCEPHALUS*)

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KEYWORDS: sperm whale, *Physeter macrocephalus*, Gulf of California, sex determination, Gulf of California.

In sperm whales, *Physeter macrocephalus* (Linnaeus, 1758), sexual segregation is evident in both the differential geographic distribution of the sexes and the species' social organization (Rice, 1989). Females and their dependent young are mostly found in tropical and subtropical waters, in which they typically reside in groups of about 20 to 40 individuals (Whitehead *et al.*, 1991a). Subadult males form non-cohesive aggregations (bachelor schools), while adult males tend to be solitary and their range extends into higher latitudes (Best, 1979; Richard, 1995). Adult males also spend periods of time in low latitudes, apparently roving among groups of females in search of mates and sometimes foraging with them (Whitehead, 1993).

Since the 18th century, sperm whales have been opportunistically sighted in the Gulf of California (Townsend, 1935; Vidal *et al.*, 1993). Adult males, groups of subadult males, and groups of females and their dependent young have been recorded there. Their distribution is apparently related to the occurrence of one of their most important prey resources, the jumbo squid, *Dosidicus gigas* (Townsend, 1935; Leatherwood *et al.*, 1988; Vidal *et al.*, 1993; Jaquet and Gendron, 2002; Ruiz-Cooley *et al.*, 2004).

It was not until the last decade, however, that systematic surveys of sperm whales were conducted in these waters (Mangels and Gerrodette, 1994[7]; Gendron, 2000; Jaquet and Gendron, 2002). In these surveys, sex determination was conducted opportunistically by visual observations. While it is generally easy to visually distinguish adult male sperm whales at sea due to their large size and distinct morphology (Best, 1979), it is difficult to reliably distinguish between adult females and non-adult males. Molecular techniques have proved to be an important tool for identifying sex in several cetacean species (Palsbøll *et al.*, 1992; Bérubé and Palsbøll, 1996; Abe *et al.*, 2001; Rosel, 2003; Morin *et al.*, 2005). Here, we report on the use of molecular techniques for the first time to determine the sex of sperm whales in the Gulf of California during fall 1999.

From August to November 1999, Ocean Alliance embarked on a four-month cruise onboard the *R/V Odyssey* in the Gulf of California, which is part of a 5-year worldwide study on pollutants in free ranging sperm whales (Godard *et al.*, 2003[8]). During this study, the *R/V Odyssey* focused search effort over steep-sided basins in the central region of the Gulf of California (Rusnak *et al.*, 1964) which have been shown to be preferred habitat for sperm whales (Jaquet *et al.*, 2002). The *R/V Odyssey* spent 39 days at sea, observed sperm whales on 30 of these days, and collected tissue samples using a projectile biopsy system on 28 days. In order to minimize potential disturbance to the whales due to close approach of the vessel, the *R/V Odyssey* has a platform near the bow that projects laterally for eight meters. Biopsy arrows are deployed from this platform so the minimum distance required between vessel and whale can be achieved. Biopsy darts used were 40mm long and 8mm in diameter. The stainless steel cylindrical punch was washed with soap, sterilized in alcohol and rinsed in de-ionized water before use. Arrows fitted with a compressed foam stopper (Barrett-Lennard *et al.*, 1996) were fired at a range of 10 to 20m from a 68kg pull, compound crossbow (Barnett RC 150). Biopsy samples were usually collected from the flank of the animal below the dorsal fin, and the region of each dart was recorded. The floating dart was recovered with a dip net.

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Skin samples were stored in 20% dimethyl sulfoxide (DMSO) solution saturated with NaCl (Amos and Hoelzel, 1991) and then transferred to a –80°C freezer. Genomic DNA was isolated using a salting out method based on a modification described by Aljanabi *et al.* (1997). Gender was determined using a multiplex polymerase chain reaction (PCR) method (Saiki *et al.*, 1988), modified from Richard *et al*. (1994), in which both SRY (male determining factor) and keratin (used as positive PCR control) genes were amplified (Table 1). PCR amplifications of biopsy samples from the Gulf of California were performed together with two positive controls of a male and female obtained from stranded sperm whales in which sex was determined by physical examination (Southwest Fisheries Science Center-SWFSC Tissue Archive, La Jolla, CA, USA).

The PCR was performed in a programmable thermal cycler (PTC-100, M J Research, Inc.) with an initial denaturation at 92°C for 2 minutes, followed by 45 cycles of 94°C for 30 seconds, 58°C for 45 seconds, 72°C for 45 seconds, and 3 minutes at 72°C for final extension. PCR products were loaded on a 2% agarose gel and electrophoresed at 85 volts for 30 minutes. The keratin product fluoresced under UV light at ~311 bp and the SRY (male) product at ~152 bp. Thus, males were revealed by two bands on the gel at ~311 bp and ~152 bp and females were identified as one band at ~311 bp.

Sperm whales were found around San Pedro Martír Island, in the Guaymas Depression area and along the Carmen and Farallon Depresions (Figure 1). These results are consistent with sperm whale aggregations reported by Jaquet *et al.* (2002) from May to July of 1998 and 1999 and extend these observations into the late summer and fall, September through November.

Two-hundred sixteen biopsy samples were collected. Of these, 192 successfully amplified for sex determination; 160 samples were determined to be female and 32 were male (Table 2). Sex results were plotted on a bathymetric map using Geographic Information System (GIS) software (Arc View GIS version 3.2). Mixed groups were mainly found in waters where the depth reaches 800-1000m followed by waters where the depth reaches 1600-1800m. Previous work reports that mixed groups usually stay in offshore waters where they feed on meso and bathypelagic squid (Whitehead *et al.*, 1991*a*; Best, 1999). The adult males in this study were found in water depths ranging from 350 to 1800m, which is similar to the results of several previous studies (Whitehead *et al.*, 1991*b*; Scott and Sadove, 1997; Best, 1999). Among the males, five were determined to be adults based on field observations of body size (individuals estimated to be about 13-15m in length) following the body size classification scheme proposed by Best (1979) and Whitehead (1993). The remaining males were categorized as "non-adult" and thus included samples taken from all smaller size classes.

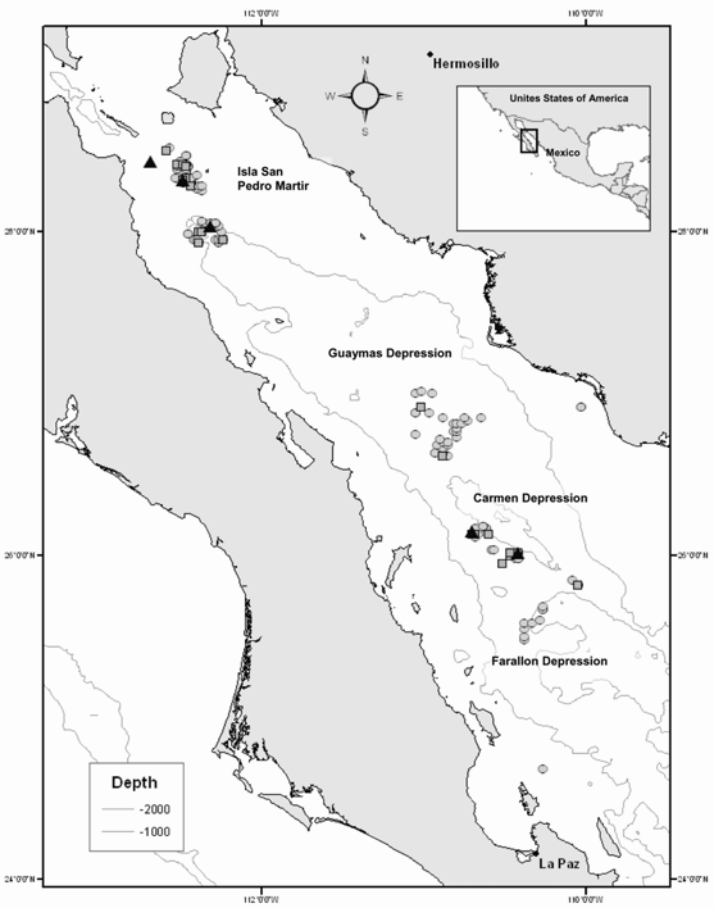

Figure 1. Sperm whales sampled in Gulf of California from August to November 1999. (●) females, (■) non adult males, (▲) adult males, (●) cities.

Table 1. Primers used in the genetic sex determination of Gulf of California sperm whales.

PRIMER	SEQUENCE	REFERENCE
Sry PMF	5′ CATTGTGTGGTCTCGTGATC 3′	Richard *et al*. (1994)
Sry PMR	5′ AGTCTCTGTGCCTCCTCGAA 3′	Richard *et al*. (1994)
KF	5′ AGATCAGGGGTTCATGTTTCTTTGC 3′	J. Hyde, SWFSC, pers. comm.
KR	5′ TTTACAGAGGTACCCAAGCCTAAG 3′	J. Hyde, SWFSC, pers. comm.

No "bachelor schools" were found. Of the five adult males identified, none were solitary and all were sampled with at least one female and sometimes many more (Table 2). This result combined with field observations of calves and small immature animals swimming together with the sampled animals are consistent with the findings of Jaquet *et al.* (2003) who suggest that the Gulf of California is likely to be a breeding ground for sperm whales.

Molecular tools have been used to determine the sex of individuals and population identity of a number of large whale species in the Gulf of California (*e.g.* Bérubé *et al.,* 2002; Croll *et al.,* 2002; Gendron, 2001; Enriquez-Paredes, 2005; Gonzalez-Peral *et al.,* 2006). This information is useful for building a more complete picture of the ecology of wild populations and for determining critical habitat when designing conservation strategies. For example, the genetic distinctiveness of fin whale (*Balaenoptera physalus*) samples from the Gulf of California led the authors to propose a separate conservation unit with special managements needs (Bérubé *et al.,* 2002). In addition, the merging of genetic, acoustic, and field data on Gulf of California fin whales led Croll *et al.* (2002) to suggest that this species uses the region as a foraging and display ground. Long-term field surveys of blue whale (*Balaenoptera musculus*) mother-calf pairs in the Gulf of California support the idea that this area is also a critical nursing and calving habitat for the species and where mating possibly occurs (Gendron, 2001; Enriquez-Paredes, 2005). The sex, pattern of distribution and high haplotypic genetic diversity among humpback whale (*Megaptera novaeangliae*) samples in the Gulf of California implies that this region is an important breeding ground for the species in the North Pacific (Gonzalez-Peral *et al.,* 2006).

As a subtropical area with high primary productivity levels (Zeitschel, 1969; Álvarez-Borrego *et al.,* 1991), the Gulf of California supports a diversity of temperate and warm water cetacean species which coexist in this dynamic, rich and productive marginal sea (Urbán, 1993[9]; Alvarez-Borrego, 2002). Previous studies (Bérubé *et al.,* 2002; Croll *et al.,* 2002; Gendron, 2002; Enriquez-Paredes, 2005; Gonzalez-Peral *et al.,* 2006) suggested the Gulf of California is a critical area for large whale conservation. The research presented here is the first effort to apply large scale genetic data from sperm whale samples in the Gulf of California and confirms that this region is an important habitat for male and female sperm whales.

Table 2. Number of sperm whales sampled at the same time and place and the sexes of the individuals sampled together (designated as the number of females/males). Total group size was not noted by *R/V Odyssey*. F= female, M= non-adult male, MA= adult male.

NUMBER OF INDIVIDUALS SAMPLED TOGETHER	2	3	4	5	6	7	8	9	10	>10
Sexes of the individuals sampled together	2F	3F	4F	5F	5F/ 1M	6F/ 1M	8F	5F/ 3M/ 1MA	10F	21F/ 1M
	2F	3F	3F/ 1M	4F/ 1M	5F/ 1M	5F/ 2M	7F/ 1M	7F/ 1M/ 1MA		11F/ 3M
	2F	3F	3F/ 1M		4F/ 2M		3F/ 5M			18F/ 1MA
	2F									8F/ 3M/ 1MA
	1F/ 1MA									

Acknowledgements

We are grateful to the Ocean Alliance whose financial support and generous sharing of tissue samples made this research possible. Our thanks also go to the crew of the *R/V Odyssey*. This research was conducted under permit No 4903 from Mexico's Secretaria de Medio Ambiente Recursos Naturales y Pesca. Samples were exported to the USA under a scientific CITES permit No. 821387. We are grateful for the expertise and financial support of the Molecular Ecology Laboratory at SWFSC, in particular: Phillip Morin, Carrie Le Duc, Kelly Robertson, Aimee Lang, Kelly Coultrup, John Hyde and Janet Lowther. Karen Evans shared the positive control samples of stranded specimens. For assistance with the GIS mapping, we thank Richard Crosgrove (SWFSC). For their special support and encouragement, we thank Lorenzo Rojas Bracho (Instituto Nacional de Ecologia), Iain Kerr, Kim Marshal and Josh Jones (Ocean Alliance), Ira Fogel (CIBNOR) and Sergio Flores, Clara Perez and Maribelle Cruz (UABCS). Carlos Olavarria and an anonymous reviewer provided thoughtful comments on the manuscript.

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Received 18 July 2006. Accepted 1 December 2006.