# **Southeastern Pacific humpback whales (*Megaptera novaeangliae*) and their breeding grounds: Distribution and habitat preference of singers and social groups off the coast of Ecuador**

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## ABSTRACT

Understanding the distribution, habitat preference and social structure of highly migratory species at important life history stages (*e.g.,* breeding and calving) is essential for conservation efforts. We investigated the spatial distribution and habitat preference of humpback whale social groups and singers, in relation to depth categories (<20 m, 20 - 50 m, and >50 m) and substrate type (muddy and mixed) on a coastal southeastern Pacific breeding ground. One hundred and forty-three acoustic stations and 304 visual sighting were made at the breeding ground off the coast of Esmeraldas, Ecuador. Spatial autocorrelation analysis suggested singers were not randomly distributed, and Neu's method and Monte Carlo simulations indicated that singers frequented depths of <20 m and mixed substrate. Singletons, and groups with a calf displayed a preference for shallower waters (0 to 20 m), while pairs and groups with a calf primarily inhabited mixed bottom substrates. In contrast, competitive groups showed no clear habitat preference and exhibited social segregation from other whales. Understanding the habitat preference and distribution of humpback whales on breeding and calving grounds vulnerable to anthropogenic disturbance provides important baseline information that should be incorporated into conservation efforts at a regional scale.

**Key words:** Song, spatial distribution, habitat preference, depth, sea floor substrate, humpback whale, *Megaptera novaeangliae*, Southeastern Pacific.

## INTRODUCTION

Humpback whales undertake extended transoceanic migrations from high latitude feeding grounds to tropical and subtropical breeding destinations located close to coastal regions (Acevedo *et al*. 2007). In the Southeastern Pacific, humpback whale concentrations are commonly observed in shallow water at the seasonal breeding grounds located in Peru, Ecuador, Colombia, and Panama (IWC Group G: review Flórez-González *et al*. 2007). This population migrates from summer feeding grounds located along the Antarctic Peninsula and Magallanes Channel (IWC 2006; Area I) (Gibbons *et al*. 2003, Acevedo *et al*. 2007, Rasmussen *et al*. 2007) to the breeding grounds, potentially through offshore waters (Félix and Guzmán 2014). The Southeastern Pacific humpback whale population requires additional baseline information (*e.g.,* migration routes and behavioral ecology) to ensure that adequate conservation measures can be implemented (Flórez-González *et al*. 2007, Stimpert *et al*. 2012, Acevedo *et al*. 2013).

Off the coast of Esmeraldas, Ecuador, the Galera-San Francisco marine reserve was established in 2008 to protect part of the breeding grounds for the Southeastern Pacific population of humpback whales (Group G), and the marine biodiversity within it (Denkinger *et al*. 2006). In addition, the Comisión Permanente del Pacífico Sur (Permanent Commission for the Southern Pacific, or CPPS) adopted a marine mammal action plan to protect key habitats for whales (Flórez-González *et al*. 2007). However, sound contamination which is increasing worldwide, is not part of the plan and could impact the vocal communication of whales. Given the suite of anthropogenic pressures faced by whale populations, it is important to understand the acoustic behavior, spatial distribution of social groups, and habitat preference of humpback whales off the Ecuadorian coast. Investigating environmental parameters and underwater sound pollution is crucial to support long-term conservation and management strategies for humpback whales in the region.

Different habitat characteristics (*e.g.,* temperature, depth, and bottom structure) can influence the geographical distributions of humpback whales when they migrate or utilize breeding grounds (Rasmussen *et al*. 2007). Recent studies have shown that sea surface temperature (SST) and depth are important indicators in understanding whale spatial distribution and habitat preference, and for predicting the extent of breeding, nursery and calving habitat (Smith *et al*. 2012, Guidino *et al.* 2014). The availability of different substrate types and depth ranges has been used to develop predictive habitat models with the goal of identifying core breeding areas for humpback whales (see Smith *et al*. 2012). Therefore, local geographic, environmental, and oceanographic parameters can assist in explaining habitat preferences and spatial distributions on the breeding grounds of large whales (Hooker *et al*. 1999, Rasmussen *et al*. 2007, Smith *et al*. 2012).

Acoustic behavior ('song') is recorded primarily on winter breeding grounds (Payne and McVay 1971, Payne and Payne 1985, Smith *et al*. 2008, Garland *et al*. 2011), but song production has also been reported during migration and on summer feeding grounds (Vu *et al.* 2012, Stimpert *et al*. 2012, Garland *et al*. 2013*b*). Song is a complex, stereotyped, and repetitive display produced by male humpback whales (Payne and McVay 1971, Payne and Payne 1985, Frankel *et al*. 1995). Although song function still is a subject of debate, the most accepted hypotheses are that song functions as a sexual advertisement to females, and/or is directed at males to mediate male-male interaction or for male social sorting on the breeding grounds (see Tyack 1981; Darling *et al*. 2006, 2012; Smith *et al*. 2008).

Overall, singers appear to be concentrated in relatively shallow coastal waters and over distinct substrate types. Singers typically sing while stationary, but are also capable of singing when they are moving (Frankel *et al*. 1995) and migrating (Clapham and Mattilla, 1990, Noad and Cato 2007). Songs have been recorded most often in shallow water (between 15 and 55 m depth), and over sandy substrates and flat seafloors (*e.g.,* Noad *et al*. 2004, Cartwright *et al*. 2012). Shallow water may overlay other factors such as seafloor composition; for example, singers in the West Indies are more often encountered over smooth substrates than any other substrate type (Whitehead and Moore 1982). Song occurrence may depend on additional acoustic factors relating to sound transmission and propagation in different habitats (Mercado and Frazer 1999). In northwestern Hawaii and the central American Pacific coast, singers have been recorded in substantially deeper waters (Frankel *et al*. 1995, Rasmussen *et al*. 2011).

The distribution of social groups may be the result of a number of factors including geographical and oceanographic requirements, social organization, female presence, and human interactions (Ersts and Rosenbaum 2003; Darling *et al*. 2006; Smith *et al*. 2008, 2012; Cartwright *et al*. 2012). For example, in Brazil, Ecuador, and Hawaii, mother-calf pairs commonly prefer shallower waters less than 20 m in depth (Smultea 1994, Martins *et al*. 2001, Félix and Haase 2005, Craig *et al*., 2014), whereas singletons, pairs, competitive groups, and singers have been observed in depths of 10 to 60 m (Martins *et al*. 2001, Oviedo and Solís 2008, Guidino *et al.* 2014). In contrast, at wintering grounds located off the central American Pacific coast and the Hawaiian Islands, mother-calf pairs and singers were commonly observed in offshore waters (*e.g*., up to 200 m) (Frankel *et al*. 1995, Rasmussen *et al*. 2011, Cartwright *et al*. 2012). Here, we investigate the spatial distribution, habitat preference and social stratification of singers (using high quality song) and other whale groups within a western South American breeding ground (Ecuador) that is at risk from expanding port activities and tourism.

## METHODS

*Study area*

Northern Ecuador is one of the multiple breeding locations for humpback whales that migrate along the west coast of South America (Group G) (IWC 2006). Our study area off the Esmeraldas coast extends from the Esmeraldas River (N 0º59'54.1'', W 79º38'37.7'') to Punta Galera (N 0°49'10.15', W 80°02'55.67") (Fig. 1). We surveyed 1,988 $km^2$ of the continental shelf to the 200 m contour, approximately 70 km offshore. The study area (Bajos de Atacames) is tropical, due to the influence of the Panama current and Equatorial Countercurrent (Murphy 1938). The seabed structure is composed of areas with hard substrates, mixed bottoms composed of sand and rock, rock walls (mixed substrate 36%), and soft bottoms containing muddy channels (soft bottom 64%), ranging in depths from 10 to 60 m, with deeper waters (1,000 m) off the continental shelf (Denkinger *et al*. 2006).

*Data collection*

Boat-based humpback whale acoustic surveys were conducted for 32 d, between June and August 2012 (Table 1). During the surveys we travelled at a speed of approximately 20 km/h on randomly distributed routes covering the entire research area from South to North and from shallow waters to >50 m depth in the West. We conducted a standardized *ad hoc* acoustic sampling effort every 25 to 30 min ($n$ = 32 acoustic recording and visual surveys) (Fig. 1) covering different parts of the study area each day. We sampled at acoustic stations with a minimum of 10 km distance between each other in order to avoid spatial autocorrelation.

Songs were recorded when a clear pattern of sound units were produced by a singer. The songs were classified as good to very good (high quality) signal-to-noise ratio (SNR) based on a loud, clear song of a single individual and the ability of an analyst to identify all units present and follow the theme pattern to identify song structure (*e.g.,* Garland *et al.* 2011, 2012, 2013*a, b*). When high quality song was present it was recorded for 30 min or more. Other recordings, lasting from 5 to 15 mins, were carried out to confirm recording quality or the absence of song. The locations of recordings with high quality, clear song were included in spatial and habitat preference analysis for singers.

During each song recording and when whales were sighted, information on sea state, geographic position, group size, presence of calves, underwater sounds, and behavior was noted. Acoustic recordings were made with an H2a-XLR omnidirectional hydrophone (sensitivity of -180 dBV/uPa +4 dB, from 20 Hz to 100 kHz) and a Tascam DR-40 tape recorder (WAV files, 16 bit, 44.1 kHz). Songs were recognized from the distinctive species-typical harmonic sounds, long vocalization times, and repeating patterns (Payne and McVay 1971).

Social groups and group membership were identified through synchronized behavior and individuals within two body lengths of each other (Whitehead 1983, Weinrich 1991). The groups were identified as: singleton, pairs, mother-calf pair, mother-calf-escort group, or competitive group (see Tyack and Whitehead 1983). Singers were presumed to be male, and the closest animal to a calf was presumed to be its mother, thus female (*e.g.,* Darling *et al*. 2006).

*Spatial analyses*

Recording locations with high quality song and visual whale sightings were mapped and displayed in ArcMap software on a chart with information on depth ranges and bottom structure (see Denkinger *et al*. 2006). We grouped depth values, which were used to explore the spatial distribution and habitat preference of each whale group. Depth was divided into three categories: <20 m, 20 - 50 m, and >50 m, while substrates were classified as mixed substrate (composed of sand and rock, rock walls) and soft bottom (muddy channels). Recordings with high quality song and group locations sighted within 100 m of the boat were considered as independent events (MacLeod *et al*. 2007). The GPS position was used as a proxy for animal position for all spatial analyses ($n$ =154 social groups matched to depth categories, and $n$ = 137 to substrate categories). All spatial analyses and distribution maps were analyzed using the Spatial Statistics toolbox of ArcMap, GIS 10.0.

*Singer locations*

To analyze spatial distribution and habitat preference of singers, the locations of recordings with clear, high quality songs were included in spatial analysis. The majority of potential singers in this study were not visually identified (2 of 33 were identified during recording); however, intense and low frequency sounds ("moans") that were present in all recordings, together with the presence of whales close by (within a radius of 800 m), allowed us to empirically estimate their position (see Cato *et al*. 2001). Therefore, we assumed that locations of recordings from singers with high quality song were likely to be within 1 km of the boat in order to estimate a potential location for spatial analysis (Fig. 2). We analyzed the overall spatial autocorrelation of high quality song recordings using a global Moran's Index to determine a clustered, dispersed, or random spatial distribution (Lloyd 2007). We used song location and song quality to analyze the broad spatial patterns of singers within the study area (Getis and Ord 1992). In addition, a basic Monte Carlo Model simulation was carried out to evaluate the probability of high quality song occurrence at each depth level and substrate (Table 2). From our model, 1,000 random iterations and ten sample repetitions were carried out for each discrete variable (Table 3) (Raychaudhuri 2008), while Neu's Index analysis was used to explore the possibility of habitat preferences.

*Social group distribution*

Data from mother-calf and mother-calf-escort groups were combined into a single category, called groups with a calf, due to data constraints (small sample size). An exploratory Nearest Neighbor Analysis (NNA) using the cumulative spatial distribution of all humpback whale group compositions and within social groups was carried out to explore the distributions of social groups (uniform, random or clustered) within the study area (Table 4). The NNA is expressed as a ratio of the observed distance divided by the expected distance (based on a random distribution with the same number of data points) (Johnston *et al*. 2001, Manly *et al*. 2002, Mitchell 2005).

*Habitat Preference*

Neu's method was used to detect habitat preference by singers and different social groups for particular depth ranges (0 - 20 m, 20 - 50 m, >50 m) and substrate types (muddy or mixed substrate). We used a chi-squared goodness-of-fit test of numbers of high quality songs (singers) obtained by a random Monte Carlo model and social group crude data to determine whether the utilization (frequencies) of depth and substrate type was proportional to their availability (Neu *et al.* 1974; Randall and Steinhorst 1984). We then created Bonferroni confidence intervals to calculate the true proportion of utilization and expected values for recording song from singers and social groups. We used confidence intervals (CI 95%) to determine whether whales exhibited "no preference" (the expected value was above the confidence intervals), "neutral" (the expected value was inside the confidence intervals) or "preference" (the expected value was below the confidence intervals) (see Cartwright *et al.* 2012, Guidino *et al.* 2014).

## RESULTS

*Song recordings*

Song was common in the study area and routinely recorded (5 of 143 recordings did not detect song) through sampling in the three distinct depth categories <20 m, 20 - 50 m, and >50 m. Moran's Index spatial autocorrelation analyses suggested that the location of high quality song recordings ($n$ = 33) and thus singers, were not randomly distributed in our study area (Moran's Index = -0.0231, expected Index = -0.0312, $Z$ - Score = 0.2388, $P$ < 0.811 3, IC = 90%); singers displayed a dispersed distribution. Accordingly, the Monte Carlo simulation and Neu's method (Table 5, 6; Fig. 3) indicated that high quality song was more likely to occur in depths of <20 m and over a mixed substrate. For depths between 20 and 50 m, singers showed a neutral or 'no preference' pattern; however, taking into account the availability of habitat on this breeding ground, singers do not appear to prefer depths exceeding 50 m (Table 5, 6).

*Visual sightings*

A total of 579 whales were observed in 304 sightings with a group size ranging between one and eight individuals (mean group size = 1.90, SD = 1.12). Of the 304 observations, only groups sighted within 100 m of the boat ($n$ = 154) were included in the spatial and habitat preference analyses. Singletons (42 %) and pairs (33 %) were the most commonly observed groups, followed by groups with a calf (13%) and competitive groups (12 %).

Within the study area, the overall distribution of humpback whales (among all social groups) was clustered over certain depth and substrate composition ranges (NNA index value = 0.72, Z-Score = -6.55, $P$ < 0.01). However, within social groups, competitive groups showed a random distribution, whereas singletons, pairs, and groups with a calf showed a clustered distribution over particular depths and substrate types (Table 4; Fig. 2). The clustered distribution within groups was not statistically significant ($P > 0.05$), except for pairs ($P < 0.01$, index value = 1.026) (Table 4). Spatial analysis indicated a clustered distribution with a slight segregation of social group types (*i.e.,* groups with a calf, pairs, and singletons) across the study area (Fig. 2).

All social groups (singletons, pairs, groups with a calf, and competitive groups) were sighted in depths of less than 20 m, and the majority of sightings for each social group were over a mixed bottom type (Fig. 2). Neu's method indicated that expected depth values were significantly different from observed values for singletons and groups with a calf ($P < 0.05$). Singletons and groups with a calf showed a significant preference for shallower water (<20 m), while pairs appear to present a neutral or no particular preference to depth (Table 5). Pairs and groups with a calf showed a particular preference for mixed bottom substrates, supported by the significant difference in expected and observed values for substrate type ($P<0.05$; Table 6). In comparison, the chi-squared goodness-of-fit test showed competitive groups displayed no preference towards any particular substrate or depth (Table 5, 6).

## DISCUSSION

The prevalence of song, young calves, pairs, and competitive groups indicates that the coast of Esmeraldas represents an important breeding ground for the Southeastern Pacific population (Group G). Little is known about the behavioral ecology of humpback whales at breeding grounds within the region. The spatial distribution and habitat preference information of humpback whales on this important breeding and calving ground, provides important baseline information that should be incorporated into conservation efforts for mitigating anthropogenic disturbance at a regional scale.

Little is known of the distribution and acoustic behavior of singers in the Southeastern Pacific. The present study routinely recorded song throughout the study area. Singers are typically stationary while singing on the breeding grounds, although they are clearly capable of singing while moving (such as on migration) (Noad and Cato 2007). Most singers were not accurately geo-referenced in our study; therefore, we estimated a range of possible locations, based on the audibility of the intense song (moans: clear low-frequency sounds heard often) (Cato *et al*. 2001). Moran's Index indicated that singers displayed a tendency towards a dispersed distribution. Previous studies suggest that humpback whale singers can be found spaced between other singers, with a higher density of singers in nearshore waters (*e.g.,* Tyack 1981, Frankel *et al*. 1995). The explorative spatial analysis detected similar patterns in our study. Singers displayed a significant habitat preference to mixed substrates and shallow water <20 m (Table 5, 6). This may be the result of uneven sampling effort as most effort was focused in shallower water. However, 40% of the acoustic sampling effort (*n* =143 samples) was in deeper water yielding sufficient opportunity to record high quality song from singers throughout the Esmeraldas study area including deeper waters.

At wintering grounds off the coasts of Central America, singing humpback whales have showed a different distribution pattern. Singers have been more commonly found in deeper depths of 30 to 50 m, but also occur further offshore at 50 to 100 m depth (Rasmussen *et al*., 2011). Further, singers and other social groups (*e.g.,* pairs, singletons, mother-calf pairs, and competitive groups) may present an overlapped and clustered distribution, as observed in Osa Peninsula, Costa Rica (Oviedo and Solís 2008).

Whitehead and Moore (1982) reported that singers in the West Indies were generally found over smooth bottoms and shallow, flat bottom substrates. The location and the undertaking of singing may be influenced by a number of factors including social, temporal, spatial, and acoustic requirements (*e.g.,* sound transmission and propagation in different habitats). For example, smoother substrates may be more absorptive to sound energy (song), while sandy substrates are more reflective potentially improving sound propagation in this habitat (Mercado and Frazer 1999). Singers in our study displayed a preference for shallow water and mixed substrates. Similar trends have been observed at North Stradbroke Islands on the east coast of Australia (Cato *et al*. 2001, Noad *et al*. 2004) and off the northwestern coast of the 'Big Island' of Hawaii, where singers display a slight preference for flat and sandy bottoms (Cartwright *et al*. 2012). However, singers are also found in deeper water (Frankel *et al*. 1995, Rasmussen *et al*. 2011). These oceanographic and topographic features may influence singer distribution and this preference may vary geographically among breeding grounds.

In addition, interactions of singers with surrounding social groups are likely to affect their location (Whitehead and Moore 1982, Smith *et al*. 2008). Singers may simply be broadcasting their songs in areas of higher whale density, using these core areas to increase the probability of being heard. This aggregative behavior in higher density areas may explain their wider distribution throughout the breeding ground in our study, whereas at a finer scale singers are located in the mid-depth range (10 - 50 m) and over mixed substrate frequented by females with or without a calf. Smith *et al*. (2008) found that singers could join a female with a calf, supporting an intersexual function to song. However, singers could also attract rival male competitors, potentially placing the singer at a disadvantage if this yielded competitive interactions or hampered the biological effectiveness of each singer.

The spatial distribution and habitat preference of humpback whales on other wintering grounds indicates that social group stratification and clustering occurs based on geographic parameters (Rasmussen *et al*. 2007, Bruce *et al*. 2014). From our limited data, groups with a calf (mother-calf pairs and mother-calf-escort groups) displayed a clustered distribution, and showed a preference for shallow water less than 20 m (79%), and mixed substrates (70%), which may provide additional shelter and protection of their young from prospecting males (*e.g.,* competitive groups). Off West Maui, Hawaii, females with a dependent calf occurred most often in shallow water to avoid unwanted male presence, suggesting a maternal strategy (Craig *et al*. 2014). In Jervis Bay, southeastern Australia, mother-calf pairs are found in areas with a gentle slope and calm water (from 15 to 20 m in depth and up to 20 km from shore) (Bruce *et al*. 2014). However, at Au´au Channel, Hawaii, groups of adults appear to avoid water depths of less than 40 m and more than 80 m, while mother-calf pairs prefer depths between 40 and 60 m, and rugged topography (Cartwright *et al*. 2012). It is possible that other factors such as human activities (*e.g.,* recreational fishing, level of navigation, whale watching, and shipping traffic) are impacting the distribution of humpback whales.

Pairs are associations commonly formed between sexually mature males and females with the intention of mating (Tyack and Whitehead 1983, Mobley and Herman 1985, Clapham 1996). They have been frequently reported at important breeding grounds on the eastern coast of Australia (*e.g.,* Brown *et al*. 1995, Burns 2010) and recently, at a breeding ground in northern Peru, Southeastern Pacific (Guidino *et al*. 2014). These mating pairs may be dynamic during the breeding season; other males may join the pair (Andriolo *et al*. 2014), which could explain why they didn't show any depth preference but a clear preference to mixed bottoms, where high frequencies of singleton whales occurred on this breeding ground.

Competitive groups displayed a more dispersed pattern and, according to Neu's index, this group indicated no preference for a specific substrate type or depth. Males within competitive groups are attempting to gain mating access to a female (Mobley and Herman 1985) and are unlikely to be selectively focused on a certain habitat type. Females within these groups, with or without a calf, are likely to be actively attempting to dislodge escorts and may be moving erratically with little regard for their location. Competitive groups were also commonly observed in offshore waters in our study (>50 m), where it may be easier for the female to maneuver, and males to engage in agonistic interactions, than in shallow water (Erst and Rosenbaum, 2003), where movements may be constrained by seabed structures such as coral heads and large rocks (Whitehead and Moore 1982).

The spatial distribution and habitat preference of humpback whales on wintering grounds in the Southeastern Pacific is sparingly reported. Our results indicate that singers, groups with a calf, and singletons showed a significant preference for shallow waters (<20 m), while singers, pairs and groups with a calf preferred mixed substrates. Therefore, nearshore waters along the coast of Esmeraldas (similar to other breeding and migratory locations in the Southeastern Pacific and central American Pacific) (Félix and Haase 2005, Oviedo and Solís 2008, Guidino *et al.* 2014) are particularly important to mothers and calves. Information on the acoustic behavior, distribution of social groups and natural habitat preferences in relation to environmental characteristics of humpback whales from long-term surveys and acoustic monitoring will allow definition of key habitats for this population, and help develop efficient conservation management of humpback whales in this marine sanctuary.

## CONCLUSIONS

Spatial analyses revealed singers displayed a dispersed distribution and a preference for shallow waters and a mixed substrate. Singers, singletons, pairs, and groups with a calf had a preference for shallow waters, unlike competitive groups, which showed a slight social segregation within this reproductive area. All behavioral and acoustic data indicated the coast of Esmeraldas is an important breeding ground through the presence of song, the formation of competitive groups actively engaged in antagonistic behaviors in pursuit of a female, and finally, the presence of young calves. This study provides important baseline information on the spatial distribution and habitat preference of humpback whales using social structure and acoustic behavior at this breeding ground of the Southeastern Pacific population (Group G). Results from this study should be incorporated into policy to establish priority areas for protection, management, and conservation measures for Ecuador's waters.

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## LITERATURE CITED

Acevedo, J. A., K. Rasmussen, F. Félix, *et al*. 2007. Migratory destinations of humpback whales, *Megaptera novaeangliae* from the Magellan Strait feeding ground, southeast Pacific. Marine Mammal Science 23(2):453-63.

Acevedo, J. A., D. Haro, L. Dalla Rosa, E. Secchi, J. Plana and L. A. Pastene. 2013. “Evidence of spatial structuring of eastern South Pacific humpback whale feeding grounds.” Endangered Species Research 22:33-38.

Andriolo, A., A. N. Zerbini, S. Moreira, *et al.* 2014. What do humpback whales *Megaptera novaeangliae* (Cetartiodactyla : Balaenopteridae) pairs do after tagging? Zoologia 31(2):105-113.

Bruce, E., L. Albright, S. Sheehan and M. Blewitt. 2014. Distribution patterns of migrating humpback whales (*Megaptera novaeangliae*) in Jervis Bay, Australia: A spatial analysis using geographical citizen science data. Applied Geography 54:83-95

Brown, M. R., P. J. Corkeron, P. T. Hale, K. W. Schultz and M. M. Bryden. 1995. Evidence for a sex-segregated migration in the humpback whale (*Megaptera novaeangliae*). Proceedings of the Royal Society of London. Series B, Biological Sciences 259:229-234

Burns, D. 2010. Population characteristics and migratory movements of humpback whales (*Megaptera novaeangliae*) identified on their southern migration past Ballina, eastern Australia, PhD thesis, Southern Cross University, Lismore, NSW. Pages:1-247

Cartwright, R., B. Gillespie, K. Labonte, T. Mangold, A. Venema, K. Eden and M. Sullivan. 2012. Between a rock and a hard place: habitat selection in female-calf humpback whale (*Megaptera novaeangliae*) Pairs on the Hawaiian breeding grounds. PloS one 7(5), e38004.

Cato, D. H., R. A. Paterson and P. Paterson. 2001. Vocalisation and movements of migrating humpback whales over 14 years. Memoirs of the Queensland Museum 47(2):481-489.

Clapham, P. J., 1996. The social and reproductive biology of humpback whales: an ecological perspective. Mammal Review 26 (1):27-49.

Clapham, P. J., and D. K. Mattila. 1990. Humpback whale songs as indications of migration routes. Marine Mammal Science 6:155-160.

Craig, A. S., L. M. Herman, A. A. Pack and J. O. Waterman. 2014. Habitat segregation by female humpback whales in Hawaiian waters: Avoidance of males? Behaviour 151:613-631.

Darling, J., C. Nicklin and M. Jones. 2006. Humpback whale songs: Do they organize males during the breeding season? Behaviour 143(9):1051-1101.

Darling, J., M. E. Jone and C. P. Nicklin. 2012. Humpback whale (*Megaptera novaeangliae*) singers in Hawaii are attracted to playback of similar song (L). Journal of the Acoustical Society of America 132 (5):2955-2958.

Denkinger, J., C. Suárez, A. Franco and D. Riebensahm. 2006. Proyecto ESMEMAR. Informe Final. Componente Marino. p. 4-57.

Ersts, P. J., and H. C. Rosenbaum. 2003. Habitat preference reflects social organization of humpback whales (*Megaptera novaeangliae*) on a wintering ground. Journal of Zoology London 260:337-345.

Félix, F., and B. Haase. 2005. Distribution of humpback whales along the coast of Ecuador and management implications. Journal of Cetacean Research and Management 7(1):21-31.

Félix, F., and H. Guzmán. 2014. Satellite tracking and sighting data analyses of Southeast Pacific humpback whales (*Megaptera novaeangliae*): Is the migratory route coastal or oceanic? Aquatic Mammals 40(4):329-340

Flórez-González, L., I. C. Ávila, J. C. Capella, *et al*. 2007. Strategy for the conservation of humpback whales in the Southeast Pacific. Guidelines for a regional action plan and national initiatives. Yubarta Foundation, Cali, Colombia. 106 pp.

Frankel, A. S., C. W. Clark, L. M. Herman and C. Gabriele. 1995. Spatial distribution, habitat utilization, and social interactions of humpback whales, *Megaptera novaeangliae* of Hawaii determined using acoustic and visual techniques. Canadian Journal of Zoology 73:1134-1146.

Garland, E. C., A. W. Goldizen, M. L. Rekdahl, *et al*. 2011. Dynamic horizontal cultural transmission of humpback whale song at the ocean basin scale. Current Biology 21:687-691.

Garland, E. C., M. S. Lilley, A. W. Goldizen, M. L. Rekdahl, C. Garrigue and M. J. Noad. 2012. Improved versions of the Levenshtein distance method for comparing sequence information in animals' vocalisations: Tests using humpback whale song. Behaviour 149:1413-1441.

Garland, E. C., J. Gedamke, M. L. Rekdahl, *et al*. 2013*a*. Humpback Whale Song on the Southern Ocean Feeding Grounds: Implications for Cultural Transmission. PLoS ONE 8(11):e79422.

Garland, E. C., M. J. Noad, A. W. Goldizen, *et al.* 2013*b*. Quantifying humpback whale song sequences to understand the dynamics of song exchange at the ocean basin scale. The Journal of the Acoustical Society of America 133:560-569.

Gibbons, J., J. J. Capella and C. Valladares. 2003. Rediscovery of a humpback whale, *Megaptera novaeangliae*, feeding ground in the Straits of Magellan, Chile. Journal of Cetacean Research and Management 5(2):203-08.

Guidino, Ch., M. A. Llapapasca, S. Silva, B, Alcorta and A. S. Pacheco. 2014. Patterns of spatial and temporal distribution of Humpback Whales at the Southern Limit of the Southeast Pacific Breeding Area. PloS ONE 9 (11):e112627.

Getis, A., and J. K. Ord. 1992. The analysis of spatial association by use of distance statistics. Geographical Analysis 24:189-206.

Hooker, S. K., H. Whithead and S. Gowans. 1999. Marine protected area design and the spatial and temporal distribution of cetaceans in a submarine canyon. Conservation Biology 13(3):592-602.

International Whaling Commission (IWC). 2006. Report of the Scientific Committee, Annex H, report of the Sub-Committee on other Southern Hemisphere whale stocks. Saint Kitts.

Johnston, K., J. M. Ver Hoef, K. Krivoruchko and N. Lucas. 2001. Using ArcGIS geostatistical analyst. User guide. ESRI Press, Redlands, CA.

Lloyd, C. D. 2007. Local models for spatial analysis. CRC Press (Taylor and Francis Group) Boca Raton. London, New York.

MacLeod, C. D., C. R. Weir, C. Pierpoint and E. J. Harland. 2007. The habitat preferences of marine mammals west of Scotland (UK). Journal of the Marine Biological. Association of the United Kingdom 87:157-164.

Manly, B. F. J., L. L. McDonald, D. L. Thomas, T. L. McDonald and W. P. Erickson. 2002. Resource selection by animals: Statistical design and analysis for field studies. 2nd edn. Dordrecht.The Netherlands: Kluwer Academic Publishers.

Martins, C. C. A., M. E. Morete, M. H. Engel, A. C. Freitas, E. R. Secchi and P. G. Kinas. 2001. Aspects of habitat use patterns of humpback whales in the Abrolhos Bank, Brazil, breeding ground. Memoirs of the Queensland Museum 47:563-570.

Mercado, E., and L. N. Frazer. 1999. Environmental constraints on sound transmission by humpback whales. The Journal of the Acoustical Society of America 106(5): 3004-3016.

Mitchell, A. 2005. GIS Analysis. Vol 2: Spatial measurements and statistics. User guide ESRI Press, Redlands, CA.

Mobley Jr, J. R., and L. M. Herman. 1985. Transience of social affiliations among humpback whales (*Megaptera novaeangliae*) on the Hawaiian wintering grounds. Canadian Journal of Zoology 63(4):762-772.

Murphy, R. C. 1938. Characteristics of the littoral water and weather along the Pacific Coast of Colombia and Ecuador, Eos Trans. AGU, 19(1):172-173.

Neu, C. W., C.R. Byers and J. M. Peek. 1974. A technique for analysis of utilization availability data. Journal of Wildlife Manage 38:541-545.

Noad, M. J., D. H. Cato and M. D.  Stokes. 2004. Acoustic tracking of humpback whales: Measuring interactions with the acoustic environment. Proceedings of the Annual Conference of the Australian Acoustical Society, Gold Coast, Australia, November 3–5, pp. 353-358.

Noad, M. J., and D. H. Cato. 2007. Swimming speeds of singing and non-singing humpback whales during migration. Marine Mammal Science 23:481-495.

Oviedo, L., and M. Solís. 2008. Underwater topography determines critical breeding habitat for humpback whales near Osa Peninsula, Costa Rica: Implications for Marine Protected Areas. Int. Journal of Tropical Biology. ISSN-0034-7744. Vol. 56(2):591-602.

Payne, R.S., and S. McVay. 1971. Songs of humpback whales. Science 173: 585-597.

Payne, K., and R. Payne. 1985. Large scale changes over 19 years in songs of humpback whales in Bermuda. Zeitschrift fur Tierpsychologie 68:89-114.

Rasmussen K., J. Calambokidis and G. H. Steiger. 2011. Distribution and migratory destinations of humpback whales off the Pacific coast of Central America during the boreal winters of 1996-2003. Marine Mammal Science. 28(3):267-279.

Rasmussen, K., D. Palacios, J., Calambokidis, *et al*. 2007. Southern Hemisphere humpback whales wintering off Central America: Insights from water temperature into the longest mammalian migration. Biology Letters 3(3):302-305.

Randall B., C. and R. K. Steinhorst, 1984. Clarification of a technique for analysis of utilization-availability data. Journal Wildlife Manage 48 (3):1050-1053.

Raychaudhuri, S. 2008. Introduction to Monte Carlo Simulation. Page 91-100 *in* Proceedings of the Winter Simulation Conference S. J. Mason, R. R. Hill, L. Mönch, O. Rose, T. Jefferson, J. W. Fowler eds.

Smith, J. N., H. S. Grantham, N. Gales, M. C. Double, *et al*. 2012. Identification of humpback whale breeding and calving habitat in the Great Barrier Reef. Marine Ecology Progress Series 447:259-272.

Smith, J. N., A. Goldizen, R. A. Dunlop and M. J. Noad. 2008. Songs of male humpback whales, *Megaptera novaeangliae*, are involved in intersexual interactions. Animal Behaviour 76:467-477.

Smultea, M. A. 1994. Segregation by humpback whale (*Megaptera novaeangliae*) cows with a calf in coastal habitat near the island of Hawaii. Canadian Journal of Zoology 72(5):805-811.

Stimpert, A. K., L. E. Peavey, A. S. Friedlaender and D. P. Nowacek. 2012. Humpback Whale Song and Foraging Behavior on an Antarctic Feeding Ground. PLoS ONE 7(12): e51214.

Tyack, P. L. 1981. Interactions between singing Hawaiian humpback whales and conspecifics nearby. Behavioral Ecology Sociobiology 8(2):105-116.

Tyack, P. L., and Whitehead, H. 1983. Male competition in large groups of wintering humpback whales. Behaviour 83:132-154.

Vu, E. T., D. Risch, C. W.Clark, S. Gaylord, L. T. Hatch, M. A. Thompson, D. N. Wiley, and S. M. Van Parijs. 2012. Humpback whale song occurs extensively on feeding grounds in the western North Atlantic. Aquatic Biology 14:175-183.

Weinrich, M. T. 1991. Stable social associations among whale (*Megaptera novaeangliae*) in the southern Gulf of Maine. Canadian Journal of Zoology 69:3012-3018.

Whitehead, H. 1983. Structure and stability of humpback whale groups off Newfoundland. Canadian Journal of Zoology 61:1391-1397.

Whitehead, H., and M. J. Moore. 1982. Distribution and movements of West Indian humpback whales in winter. Canadian Journal of Zoology 60:2203-2211.

## TABLES AND FIGURES

*Table 1.* Survey effort ($km^2$) by depth ranges and substrate composition.

| Categories | Study area ($km^2$) | June (5) | July (18) | August (9) | % covered | Area covered ($km^2$) |
|---|---|---|---|---|---|---|
| < 20 | 743.96 | 102.08 | 447.54 | 257.49 | 8.07 | 807.11 |
| 20-50 | 452.89 | 67.61 | 174.8 | 130.02 | 3.72 | 372.43 |
| > 50 | 790.83 | 108.69 | 130.58 | 48.11 | 2.87 | 287.38 |
| Mixed | 324904.89 | 50.18 | 254.12 | 175.22 | 4.80 | 479.52 |
| Muddy | 687090.29 | 118.78 | 412.83 | 223.23 | 7.55 | 754.84 |

() number of days research trips were carried out each month

*Table 2.* Basic Monte Carlo Model simulation with 1000 random iterations of song occurrence rates for depth and substrate.

| | Depth | Substrate |
|---|---|---|
| Sample mean | 1.342 | 1.413 |
| Standard deviation | 0.604 | 0.493 |
| Value MIN | 1 | 1 |
| Value MAX | 3 | 2 |
| Significance level | 0.050 | 0.050 |
| Amplitude C.I. | 0.037 | 0.031 |
| C.I. mean to level (1-alpha)% | 1.305 | 1.382 |

*Table 3.* Mean, standard deviation (SE), and standard error of the mean humpback whale song probability (ten sample runs) for each discrete variable (depth *vs.* substrate). C.I. 95%.

| Depth | mean (sample runs) | N | SE | SEM |
|---|---|---|---|---|
| < 20 | 727 | 10 | 0.393 | 0.124 |
| 20-50 | 211.6 | 10 | 0.121 | 0.030 |
| > 50 | 61.4 | 10 | 0.271 | 0.085 |
| Substrate | | | | |
| mixed | 616.3 | 10 | 0.116 | 0.036 |
| muddy | 383.7 | 10 | 0.116 | 0.036 |

*Table 4.* Average Nearest Neighbor analysis (NNA) within humpback whale social groups. Index values above 1 represent a uniform or ordered distribution, a value of 1 indicates a random distribution, and a value less than 1 represents a clustered distribution.

| Social groups | n | Observed Mean Distance (km) | Expected Mean Distance (km) | Z-Score | *P*-Value | Index Value | Pattern |
|---|---|---|---|---|---|---|---|
| Singletons | 40 | 0.023 | 0.023 | -0.179 | 0.857 | 0.985 | Clustered |
| Pairs | 51 | 0.014 | 0.018 | -3.395 | 0.000 | 0.768 | Clustered |
| Groups with a calf | 27 | 0.020 | 0.021 | -0.534 | 0.593 | 0.947 | Clustered |
| Competitive groups | 19 | 0.030 | 0.029 | 0.250 | 0.802 | 1.026 | Random |

*Table 5.* Habitat preference (depth) of singers and social groups of humpback whales along the north coast of Ecuador (Esmeraldas).

| Social groups | Depths | Available habitat (km²) | Expected groups (E=*n*pi **) | Expected groups proportions | Observed groups (*O*i) | Usage or observed groups (Pi) | Bonferroni 95 % C.I. range | Neu's Index | Inference | Chi-square test goodness-of-fit test |
|---|---|---|---|---|---|---|---|---|---|---|
| Singers | <20 | 743.96 | 374.29 | 0.37 | 727 | 0.727 | 0.541-0.913 | 0.642 | Preferred* | *P* <0.05, |
| | 20-50 | 452.89 | 227.85 | 0.23 | 211.6 | 0.212 | 0.041-0.382 | 0.307 | Neutral | X²= 731.22, |
| | >50 | 790.83 | 397.87 | 0.40 | 61.4 | 0.061 | -0.039-0.162 | 0.051 | No preference | df=2 * |
| Total | | | 1000.00 | | 1000 | | | | | |
| Singletons | <20 | 743.96 | 16.09 | 0.37 | 29 | 0.674 | 0.486-0.863 | 0.581 | Preferred* | *P* <0.05, |
| | 20-50 | 452.89 | 9.80 | 0.23 | 11 | 0.256 | 0.080-2.012 | 0.362 | Neutral | X²= 24.75, |
| | >50 | 790.83 | 17.11 | 0.40 | 3 | 0.070 | -0.033-0.172 | 0.057 | No preference | df=2* |
| Total | | | 43.00 | | 43 | | | | | |
| Pairs | <20 | 743.96 | 22.08 | 0.37 | 31 | 0.525 | 0.354-0.697 | 0.439 | Neutral | *P* <0.05, |
| | 20-50 | 452.89 | 13.44 | 0.23 | 19 | 0.322 | 0.161-0-483 | 0.442 | Neutral | X²= 12.34, |
| | >50 | 790.83 | 23.47 | 0.40 | 9 | 0.153 | 0.029-0.276 | 0.120 | No preference | df=2* |
| Total | | | 59.00 | | 59 | | | | | |
| Groups with a calf | <20 | 743.96 | 10.48 | 0.37 | 22 | 0.786 | 0.581-0.990 | 0.706 | Preferred* | *P* <0.05, |
| | 20-50 | 452.89 | 6.38 | 0.23 | 5 | 0.179 | -0.013-0.370 | 0.264 | Neutral | X²= 26.64, |
| | >50 | 790.83 | 11.14 | 0.40 | 1 | 0.036 | -0.057-0.128 | 0.030 | No preference | df=2* |
| Total | | | 28.00 | | 28 | | | | | |
| Competitive groups | <20 | 743.96 | 8.98 | 0.37 | 13 | 0.542 | 0.273-0.810 | 0.472 | No | *P* >0.05, |
| | 20-50 | 452.89 | 5.47 | 0.23 | 6 | 0.250 | 0.017-0.483 | 0.358 | preference | X²= 4.75, |
| | >50 | 790.83 | 9.55 | 0.40 | 5 | 0.208 | -0.011-0.427 | 0.171 | | df=2 |
| Total | | | 24.00 | | 24 | | | | | |

(*) Bonferroni confidence intervals were used to determine habitat preference, detecting significant differences between availability and usage.

(**) *n*pi = expected proportion.

Depths are used in proportion to their availability (no preference) as tested by Chi-square goodness-of-fit test.

*Table 6.* Habitat preference (substrate) of singers and social groups of humpback whales along the north coast of Ecuador (Esmeraldas).

| Social groups | Substrates | Available habitat (km²) | Expected groups (E=*n*pi**) | Expected proportions | Observed groups (*O*i) | Usage or observed groups (Pi) | Bonferroni 95 % C.I. range | Neu's Index | Inference | Chi-square test goodness-of-fit test |
|---|---|---|---|---|---|---|---|---|---|---|
| Singers | Mixed | 32404.89 | 45.04 | 0.045 | 616.3 | 0.616 | 0.520-0.712 | 0.971 | Preferred* | $P < 0.05$, $X^2 = 54.10$, df=1* |
| | Soft bottom | 687090.29 | 954.96 | 0.955 | 383.7 | 0.384 | 0.288-0.480 | 0.029 | No preference | |
| Total | | | 1000.00 | | 1000 | | | | | |
| Singletons | Mixed | 32404.89 | 1.80 | 0.045 | 24 | 0.600 | 0.515-0.685 | 0.970 | No preference | $P > 0.05$, $X^2 = 1.60$, df=1 |
| | Soft bottom | 687090.29 | 38.20 | 0.955 | 16 | 0.400 | 0.315-0.485 | 0.030 | | |
| Total | | | 40.00 | | 40 | | | | | |
| Pairs | Mixed | 32404.89 | 2.30 | 0.045 | 35 | 0.686 | 0.615-0.758 | 0.979 | Preferred* | $P < 0.05$, $X^2 = 7.08$, df=1* |
| | Soft bottom | 687090.29 | 48.70 | 0.955 | 16 | 0.314 | 0.242-0.385 | 0.021 | No preference | |
| Total | | | 51.00 | | 51 | | | | | |
| Groups with a calf | Mixed | 32404.89 | 1.22 | 0.045 | 19 | 0.704 | 0.607-0.800 | 0.981 | Preferred* | $P < 0.05$, $X^2 = 4.48$, df=1* |
| | Soft bottom | 687090.29 | 25.78 | 0.955 | 8 | 0.296 | 0.200-0.393 | 0.019 | No preference | |
| Total | | | 27.00 | | 27 | | | | | |
| Competitive groups | Mixed | 32404.89 | 0.86 | 0.045 | 11 | 0.579 | 0.454-0.704 | 0.967 | No preference | $P > 0.05$, $X^2 = 0.47$, df=1 |
| | Soft bottom | 687090.29 | 18.14 | 0.955 | 8 | 0.421 | 0.296-0.670 | 0.033 | | |
| Total | | | 19.00 | | 19 | | | | | |

(*) Bonferroni confidence intervals were used to determine habitat preference, detecting significant differences between availability and usage.

(**) *n*pi = expected proportion.

Depths are used in proportion to their availability (no preference) as tested by Chi-square goodness-of-fit test.

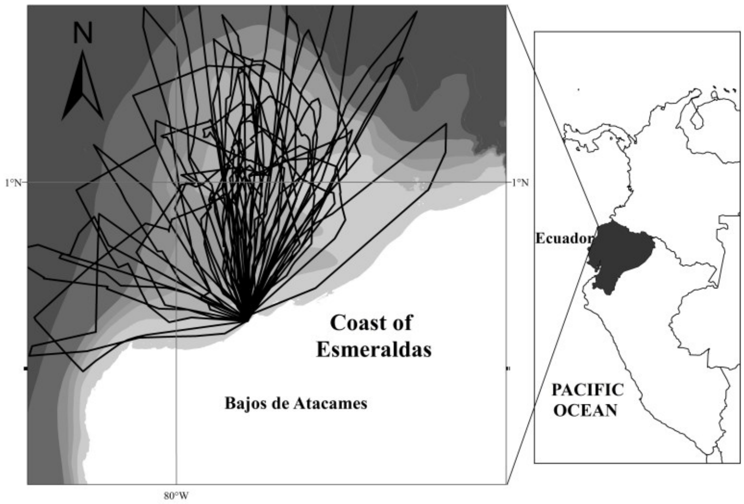


*Figure 1.* Humpback whale survey transects, the eastern South Pacific region and the study area located along the coast of Esmeraldas, Ecuador.

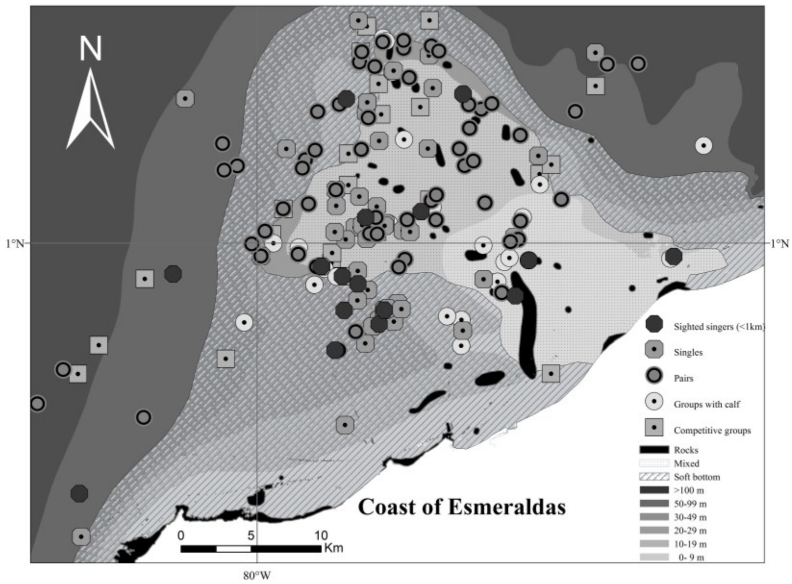


*Figure 2*. Occurrence of songs and whale social groups distribution according to bathymetry (o to >100 m) and bottom composition (mixed and soft bottom). High quality song (sighted singers < 1 km) are presented where potential singers were singing.

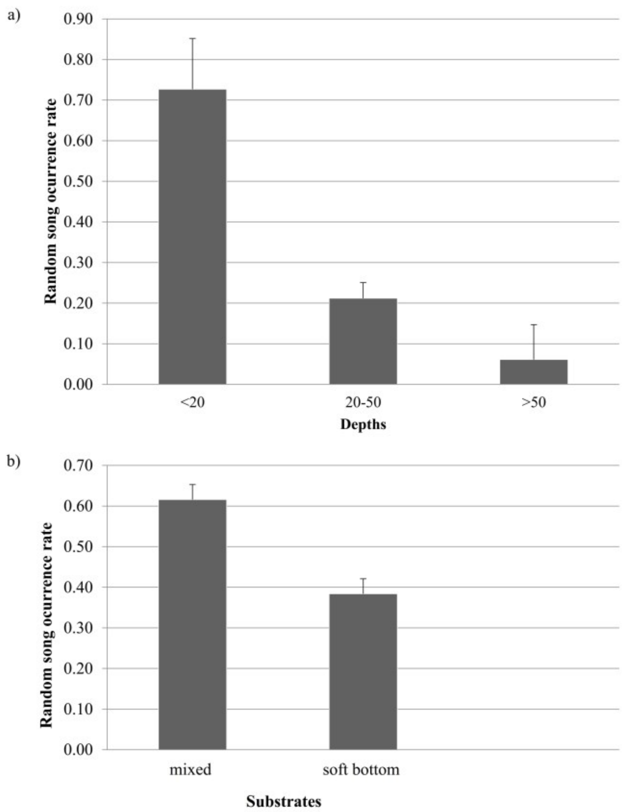


Figure 3. Random song occurrence rate (mean and error standard) from a Monte Carlo model simulation with 1000 random iterations for each depth (a) and substrate (b) and tested on ten sample runs (N=10).