EVALUATING THE ROLE OF SONG
IN THE HUMPBACK WHALE (*Megaptera novaeangliae*) BREEDING SYSTEM
WITH RESPECT TO INTRA-SEXUAL INTERACTIONS

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EVALUATING THE ROLE OF SONG IN THE HUMPBACK WHALE (*Megaptera novaeangliae*) BREEDING SYSTEM WITH RESPECT TO INTRA-SEXUAL INTERACTIONS

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Underlying any interaction between two or more individuals is some form of communication, which can be defined as the transmission of information by one individual and the use of that information by another individual in future decision-making. While natural selection shapes signals that are important for survival, sexual selection shapes signals that are important within the context of reproduction. In some cases, sexual selection may lead to the evolution of highly exaggerated traits, such as the extravagant plumage of birds-of-paradise, or the elaborate vocal performance of the common nightingale.

Humpback whales (*Megaptera novaeangliae*) are well-known for their rich acoustic display. Humpback song is the most complex among baleen whales with respect to both spectral and temporal characteristics. Although song features and the behavior of singing males have been studied since the 1970s, our understanding of the role of song within this breeding system remains incomplete.

This study investigated the role that song plays in mediating intra-sexual interactions in humpback whales. I combined passive acoustic recording and playback experiments, and developed song metrics derived from avian literature to analyze the behavior of individual singing males with respect to one another. An analysis of diel singing activity found higher numbers of singing males during nighttime hours. Analyses of song patterns revealed that males changed their song presentation while in the presence of other singers by increasing the rate at which they switch between themes, and in some cases, thematically overlapping other singers more than expected (similar to “song matching” in songbirds). Singing males were found to approach one another more than expected and previously believed. Singers responded acoustically to the presentation of song playback experiments by altering their song presentation similarly to the ways in which they responded in the presence of other singers. Taken together, these data provide evidence that male humpback whales do respond to and interact with one another while singing, and may alter their behavior to target particular individuals. The implications of these results are discussed with respect to hypotheses regarding the function of humpback song.

BIOGRAPHICAL SKETCH

Danielle Marie Cholewiak was born on November 8, 1975, in Troy, Michigan, daughter to an effervescent spirit and a rational thinker. She discovered a love of nature and wildlife in her backyard at a young age, a love which persisted throughout her developing years. The Exxon Valdez oil spill that occurred in 1989 in Prince William Sound, Alaska, stood out as a turning point in her consciousness about man's impact on the environment, and sparked her interest in the conservation of natural resources. She attended Marian High School, where she was inspired by her Environmental Science teacher and also discovered a love for foreign language study. A high school trip to the east coast of Massachusetts and her first glimpse of cetaceans on Stellwagen Bank began a lifelong fascination with marine mammals.

Danielle went on to attend the University of Michigan, where she completed two bachelor's degrees: one in Biology (through the College of Literature, Science and the Arts), the other in Resource Ecology and Management (through the School of Natural Resources). While at U of M, she completed an undergraduate thesis under the direction of Robert B. Payne, studying the begging behavior of nesting Bengalese finches. She participated in a variety of field projects, ranging from the study of riparian vegetation to the distribution of freshwater fishes, but was most fascinated with an internship in the marine mammal lab at Texas A & M University, working with Bernd Wursig and his graduate students on bottlenose dolphin and dusky dolphin behavior and vocalizations.

In 1998, Danielle began participating in a long-term field project studying humpback whale breeding behavior off Isla Socorro, México, a place that she became heavily invested in and returned to almost annually for the next eight years. She began her dissertation work in 2001 at Cornell University in the Department of Neurobiology and Behavior, under the tutelage of Dr. Christopher Clark.

Upon completing her Ph.D., Danielle will begin a post-doctoral position working with Dr. David Wiley and colleagues at the Stellwagen Bank National Marine Sanctuary.

For my family,

who taught me that anything is possible,

and that I should always follow my dreams.

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My decision to conduct my dissertation research in México was the product of several years of previous experience working on Isla Socorro with several wonderful people. México became a home-away-from-home for me over the years, and I was fortunate to find there a community who changed my life. Conducting field work on Isla Socorro was a huge effort and it would not have been possible without the help of several key people and the assistance of many enthusiastic volunteers. I would first like to acknowledge the Armada de México and the many members of the Mexican navy who made working on Isla Socorro possible. To Jeff, words cannot come close to expressing all of the thanks that I owe you. Without you, none of this would have started! Logistically, you made the array work (including the playbacks) possible, but your contribution and inspiration were and are so much broader than that. Thank you helping to bring me into my own in Socorro and for facilitating my growth on so many levels. This was a joint effort, and I only hope that what has come out of it is as worthwhile to you as it has been to me. To Jorge, you offered to help me out – perhaps without realizing what you were getting yourself into?! I know the politics and logistics were at times exasperating, but I so appreciate your support. Thank you for being my unofficial fifth committee member, for hosting me in your country, and for welcoming my collaboration. To all of my field assistants over four field seasons – Beto, Santos, Carlos, Adrian, Gloria, Tom, Jenny, Joe, Talena, Guilian, Danielle, Jon, Vero… you all comprised a great team! Thanks for your enthusiasm and hard work despite many long hours in the sun and an exhausting schedule! Friends on several vessels were a great help in transporting pop-ups from Baja out to Socorro – thank you to Solmar V and the Nautilus for helping me out. And to our friends on the Blue Chablis and Orea, I hope to see you there again.

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INTRODUCTION

Communication among individuals is essential for survival and reproduction in many animal species. One definition of communication is the transmission of information by one individual (the sender) and the subsequent use of that information by another individual (receiver) to influence future decisions (Bradbury & Vehrencamp 1998). A distinction may be made between eavesdropping, when individuals who are not the intended receivers of a signal nevertheless receive and use that information, and "true communication", which occurs between a sender and an intended receiver to the benefit of the sender (Bradbury & Vehrencamp 1998). Communication can occur across all sensory modalities, taking on the form of vocalizations, visual displays, chemical production, physical contact, or even electroreception, and is important in many different social contexts (i.e., feeding, breeding, alerting).

Communication within a breeding context

Signaling between individuals is important in mediating many types of interactions within the context of reproduction. Some of these interactions include self-advertisement, intra-sexual competition, begging, and the maintenance of pair bonds. Selection will influence the evolution and design of different types of signals depending on the species, environment, and function (Endler 1992). For example, chemical communication is widely used in many mammalian species, and females may signal reproductive status or males may signal territoriality via the use of pheromones (Alberts 1992). Visual signals may be used for short-term, rapidly modulated communication at close ranges. For example, males may mediate competition via visual patterns produced on the body (e.g., cuttlefish: Adamo &

Hanlon 1996), or by hiding or revealing colored patches (e.g., red-winged blackbirds: Hansen & Rohwer 1986). Other types of visual displays provide longer-term, more durable signals (e.g., peacock tails: Manning 1989).

Acoustic signaling may be used for both long-range and short-range interactions. Acoustic signals have the advantage that they are more easily modulated, and in some cases more localizable, than chemical signals, and may be used over larger ranges than visual signals. A variety of acoustic signals may be produced by both sexes, for example, males and females may engage in calling behavior to maintain contact, or alert others of danger (Catchpole 1982). A special form of acoustic communication which has undergone strong sexual selection in many species is the acoustic display referred to as song. The use of song as a signal is primarily restricted to males, and in many species serves in both inter-sexual as well as intra-sexual interactions.

Song

A general definition for song, derived from avian studies, is that song is "… a long, complex vocalization produced by males in the breeding season." (Catchpole & Slater 1995). This is not universally true, as not all songs are long or complex, and not only males sing (although the production of song by females is relatively rare), but this definition embodies most of the signals that are commonly recognized as song. Studies on terrestrial species have revealed two major functions of song within breeding systems: 1) attraction of mates, and 2) defense of territories against rivals.

Mate Attraction

Previous studies with songbirds have demonstrated that song functions to attract females. Suggestive evidence includes the decrease of male singing rates after pairing and the increase of singing rates after a mate is lost (or experimentally removed**,** Krebs et al. 1981). More direct evidence for female preference relative to song parameters comes from playback studies demonstrating that song characteristics (e.g., song rate, repertoire size) affect female behavior (i.e., solicitation displays, see Searcy 1992). Why might females choose mates on the basis of singing behavior or song characteristics? The evolution of male traits with respect to selection by female choice is generally thought to occur as the result of one of two main processes: 1) selection for direct benefits leads to higher female survivorship or fecundity, or 2) selection for indirect benefits leads to higher survivorship or reproductive success of the female's offspring.

Under the direct benefits hypothesis, females assess males based on song traits when those traits are linked to the quality of the benefits that males can provide the females. So, for example, if male singing rates are correlated with territory quality (such that males with higher quality territories can forage more efficiently and therefore spend more time singing), then females will prefer males with higher singing rates (Alatolo et al. 1990). Similarly, if traits such as repertoire size are indicative of male age, and if this is correlated with the ability to gain better territories, then females will prefer males with larger repertoires (Catchpole 1986).

In some species, females show preferences for song characters even though these do not seem to be related to direct benefits to the female. Village indigobirds, for example, are brood parasites who lay their eggs in the nests of other species. Males do not defend territories, and neither the male nor female provides parental care to the young. Females have been shown, however, to prefer males with higher song output (Payne & Payne 1977). The preference for traits that indicate indirect benefits is thought to arise through one of three types of selection: 1) traits that indicate "good genes", which might result in higher offspring survivorship (Hamilton & Zuk 1982),

2) traits for which males incur a cost, thereby indicating male viability (the "handicap principle", Zahavi 1975), or 3) indirect selection for an arbitrary trait that has no particular benefit other than increasing reproductive success for males who possess the trait (Fisher 1930).

Territory Defense

Many studies, notably in territorial songbirds, have also demonstrated that song plays an important role in male-male interactions. Using song to mediate interactions may allow males to assess potential rivals and avoid physical conflict by escalating or de-escalating agonistic interactions based on an individuals motivation and perception of one's own quality or competitive ability compared to that of a rival. If this is case, then there should be strong selection for song characteristics that allow males to assess one another. Therefore, song characters that are likely to be important in male-male competitions are those that are both costly and difficult to cheat. These costs may be incurred either through production or through enforcement of honesty by conspecifics (Bradbury & Vehrencamp 1998). In some cases, this could lead to the evolution of the use of the same types of song traits that may be used by females. For example, song elements that are difficult to produce, or those that indicate body size might be useful for male-male assessment. Additionally, graded song characters that allow males to predict the likelihood of escalating aggression are important in some species. Some of these song characters include: matching (Krebs et al. 1981), coordinated bout switching (Kramer & Lemon 1983), and switching rate (Simpson 1985; Molles & Vehrencamp 1999; Vehrencamp 2001). In addition, males may also use song to distinguish between familiar individuals (i.e., neighbors with whom they have established relationships) vs. unfamiliar individuals (i.e., a new arrival who may be seeking to establish a new territory (Molles & Vehrencamp 2001).

Which Taxa Sing?

The use of song is not ubiquitous among animal groups, and evaluating its prevalence is difficult due to inconsistencies in the use of the term within the literature. Definitions of song can be as broad as "…long-range acoustic signals produced mainly during the breeding season..." (Andersson 1994), to the more narrow "… a series of notes, generally of more than one type, uttered in succession and so related as to form a recognizable sequence or pattern in time." (Thorpe 1961). Applications of the term range from vocalizations that structurally fit the more narrow definition, to those that functionally fit the broader definition, with the result that the vocalizations of some species are alternately referred to as calls or song depending on the author. At its basis, the term song is derived from the long history of avian studies of passerine birds, in which many species are recognized to sing.

Song in Mammals

Among mammals, song is rare, although the use of vocal signaling on the part of males to advertise or to attract females is not (e.g., red deer: Charlton 2007; elephant seals: Sanvito et al. 2006). Only a handful of species are known to sing, including primates (gibbons: Mitani 1988), bats (Bradbury & Emmons 1974; Davidson & Wilkinson 2002), rodents (mice: Holy & Guo 2005; Miller & Engstrom 2007), and pinnipeds (Richardson et al. 1995), and cetaceans (Clark & Ellison 2004). In all of these groups, males of at least some species produce patterned sequences of vocalizations that appear to function in territory defense, mate attraction, or both. The occurrence of song is likely more common than recognized, particularly among species of certain taxa that vocalize below or above our audible frequency range.

Within cetaceans, singing appears to be restricted to baleen whales. Males of at least five of the 12 species in the group have now been shown to sing, including blue, bowhead, fin, humpback and minke whales (Payne & McVay 1971; Gedamke et al. 2001; Croll et al. 2002; Oleson et al. 2007) Among these, the functions of song have not been well-studied, but there appear to be distinct differences in the behavioral ecologies of these species that sing. For example, humpback research reveals that most singing occurs during winter months on mid-latitude calving/breeding grounds where there are essentially no food resources, while observations on blue and fin whales, species for which there are no known seasonal calving/breeding grounds, indicate that singing occurs over a broad range of latitudes often in association with food resources. Both humpback and bowhead songs are highly variable and change from season to season, while blue, fin and minke songs are highly stereotyped (Clark & Gagnon 2004; Clark & Ellison 2004).

Humpback song is the most well-studied, and is among the most complex with respect to types, frequency ranges, variability, and patterning of notes. Only bowhead whale song approaches the temporal and spectral complexity of humpback song. Because bowheads are so difficult to study, little is known about their behavioral ecology or breeding system and the selective pressures leading to the evolution of a song complexity similar to that of humpbacks remains unknown.

Humpback Whales: Breeding Behavior and Song

Humpback whales are found worldwide and undertake annual migrations that are among the longest of any mammal, as individuals travel from high-latitude feeding grounds to mid-latitude breeding grounds within each hemisphere's winter (Clapham 1996). In the North Pacific, three main breeding areas are recognized: Japan, Hawaii, and Mexico, with relatively little interchange occurring between them (Baker et al. 1986; Baker et al. 1998).

During the breeding season, individuals are primarily or exclusively fasting (Chittleborough 1965), therefore selective pressures with respect to advertising or defending food resources are unlikely to play a role within this breeding system. Humpback whales appear to have a lek-like mating system, a situation that is unique among cetaceans and uncommon among mammals, being known primarily in ungulates and bats (Clutton-Brock 1989). Social bonds between individuals are characterized by relatively brief associations (Mobley & Herman 1985), with the exception of mothers with their calves during the year of calving. Males engage in alternative mating tactics, which include consorting with females, aggressively competing to defend positions near females, and performing long, elaborate song displays (Tyack & Whitehead 1983; Clapham et al. 1992; Cerchio 2003).

The hierarchical structure of song was first described by Payne and McVay (1971). The shortest sounds are called units, which are grouped into phrases. Phrases are repeated multiple times, and a series of similar phrases is referred to as a theme. Humpback song sequences are comprised of multiple themes. An entire sequence of themes is considered to be a "song", and themes are often (though not always) repeated in the same order from one song cycle to the next. Males progressively change their songs throughout the breeding season, and males throughout an ocean basin incorporate many of these changes into their own songs (Payne et al. 1983; Cerchio et al. 2001).

The function of song within this species remains debated. Although many hypotheses have been proposed (Baker & Herman 1984; Clapham & Mattila 1990; Frazer & Mercado 2000), the predominant theories are that males sing to attract females (Winn & Winn 1978; Tyack 1981), or to establish and/or maintain dominance hierarchies between males (Darling 1983). To date, few empirical data exist by which to evaluate these hypotheses.

THESIS OBJECTIVES

When I initially began my thesis, I was interested in addressing the hypothesis that males use song to attract females. The elaborate singing displays and the fact that males are continually changing their songs suggest that song structure and dynamics are influenced by strong sexual selection. This type of rapidly evolving, culturally transmitted song is rare, known only in a handful of other species (Payne 1985; Trainer 1989). Observations of singing males revealed that males are often interrupted (or “joined”) by other males, but few studies have been able to document female behavior with respect to singers. In a small number of cases, females have been observed to associate with singing males (Tyack 1981; Medrano et al. 1994), but song playback experiments have failed to elicit approaches on the part of females (Tyack 1983; Mobley et al. 1988). The latter has largely been interpreted as evidence against the mate-attraction hypothesis, although it might be more appropriately interpreted as lack of evidence for that hypothesis. It is possible that the scarcity of observations of female behavior relative to singing males actually stems from the fact that lone, non-lactating females (i.e., those that might be most likely to assess singers) are extremely difficult to locate and follow on the breeding grounds. Additionally, all observational work with respect to interactions between singing males and other individuals has been conducted during daylight hours, while previous literature (Au et al. 2000) had suggested that males sing more at night.

Therefore, I originally embarked on a project to attach VHF radio transmitters to females, with the idea that only through detailed observation of female behavior would we begin to gather data to properly test the mate-attraction hypothesis. The project was designed to integrate continuous, autonomous acoustic recording of singing males with data collected from focal follows of radio-tagged females. Arrays of pop-ups were deployed to enable the recording and subsequent localization of singers. VHF transmitters were attached to females from a small vessel during daytime hours, and shore-based radio trackers monitored for these individuals across the acoustic array area during the night. Unfortunately, due to logistical constraints at multiple levels, the project was largely unsuccessful despite several years of effort.

I eventually switched gears and decided to re-focus my efforts on evaluating the role of song from the perspective of male-male interactions, given that I had an abundance of acoustic array data and an incredible intellectual resource within my department from which to draw ideas for developing these analyses. While singing humpback males are generally thought to avoid one another, it was completely unknown if they interact with one another acoustically. In this thesis, I draw on the body of literature developed from studies of terrestrial songbirds develop the context and methodology for examining both natural encounters of individuals as well as playback experiments. I focus on evaluating the hypothesis that males use song to mediate intra-sexual interactions. To this end, my thesis is divided into four chapters:

In Chapter One, I address the classification system used for humpback song and discuss some of the discrepancies in how this scheme is applied within the literature. I present evidence to suggest that variability in song sequence may occur more often and to a greater extent than previously reported, and suggest that this variability be informative. In comparing humpback song to bird song, I suggest new metrics for measuring the variability in humpback song sequences.

In Chapter Two, I review continuous acoustic data to test for evidence of a diel trend in singing activity. While a diel trend in song has been suggested in the literature, it has not been previously quantified. I quantify both the number of audible singers and the RMS sound pressure levels to test whether there is an increase in singing activity at night. I also discuss several hypotheses for why the observed trends in diel activity may have evolved.

In Chapter Three, I take on the question of whether singing males are acoustically interacting with one another. Using metrics derived from the avian literature, I evaluate the song sequences of male singer dyads to evaluate whether or not males change the characteristics of their songs in response to one another. Using the locations of singers calculated from the acoustic data, I evaluate the prevalent idea that singing males avoid one another spatially.

In Chapter Four, I use some of the same metrics employed in the previous chapter to address the question of how singing males respond to the playback of male song, both acoustically and spatially. I evaluate their response in light of the results of the previous chapter to answer the question of whether males respond to song playback similarly to how they respond to one another.

Taken together, these studies provide a new approach for understanding and interpreting the behavioral functions of humpback song. In many ways this approach, both experimentally and analytically, is richer because it is linked to the long history of scientific investigations of avian song function. I continue to keep in mind, however, that any analysis which focuses on the use of song from a solely intra-sexual perspective is necessarily limited in its interpretation. However, my hope is that the combination of the approach, metrics, analyses and perspective presented here will enable future studies to further advance our understanding of the evolution and use of song within the humpback whale breeding system.

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CHAPTER ONE

HUMPBACK WHALE SONG HIERARCHICAL STRUCTURE: A REVIEW, SYNTHESIS, AND DISCUSSION OF THE CONCEPT OF "SONG"[1]

> *"It has been known for a long while that humpback-whales, blackfish, devil-fish, and other species of whales sing, and that walruses and seals bark underwater, and it is believed that all animals having lungs and living in the water, as they do, have their own peculiar cry, or as whalemen express it, "sing"."* Aldrich (1889)

As recently as the 1940s, scientists did not know whether baleen whales could produce sounds. Although cetacean sounds were apparently well known to ancient whalers (Aldrich 1889), the early scientific community concluded that cetaceans were mute based upon the discovery that they did not possess vocal chords (Schevill et al. 1962). It wasn't until after the confirmation of sound production by odontocetes (Kullenberg 1947, Fraser 1947, Schevill & Lawrence 1949) that modern scientists began to realize that mysticetes also produced vocalizations.

The first documentation of humpback vocalizations within the scientific community was presented in 1952, when Schreiber (1952) recorded sounds with a "musical quality" off the island of Oahu, which he attributed to marine life. We now know that these "musical" sounds were produced by humpback whales. A decade of

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research after Schreiber's discovery uncovered the fact that humpbacks produce a wide range of vocalizations on the breeding grounds and en route to them. By 1964, their breeding sounds were becoming well known: "…the sonorous moans and screams associated with the migrations of *Megaptera* past Bermuda and Hawaii may be an audible manifestation of more fundamental vernal urges…" (Schevill 1964). Off the coast of New Zealand, acousticians were making similar discoveries, although they did not positively identify the sounds as humpback in origin. They described that a "…chorus of squeals, creaks, cries, barks, groans, and whoops… has been labeled the "Barnyard Chorus" by the laboratory staff" (Kibblewhite 1967). The same author even correctly supposed that one individual might produce all the different sounds in this chorus, though he did not attempt to confirm this. A spectrographic catalog of some of the sounds known to be produced by humpback whales demonstrated the diversity of these vocalizations (Tavolga 1968). In 1970, in a technical report written for the Naval Undersea Research and Development Center, Cummings and Philipi (1970) describe repetitive "stanzas" recorded in late December in the northwest Atlantic. Their low sampling rate precluded the detection of any sounds above 175 Hz, yet the authors were able to identify sound series that lasted 11-14 minutes, including pulses, blips and moans. They tentatively identified these sounds as the product of the North Atlantic right whale (*Eubalaena glacialis*) vocalizations; in all likelihood, they were actually listening to humpback whales (Payne & Payne 1971).

It seems that scientists working in every ocean basin were on the verge of a new discovery, but it wasn't until 1969 that these sounds were finally recognized by Roger and Katy Payne as the "song" produced by humpback whales (Anon 1969). In 1971, Payne and McVay published a pivotal paper describing the patterned, hierarchical structure of these sounds, making the first connection with bird song. Their analyses were based on recordings made by Frank Watlington off the coast of

Bermuda from 1953-1964, as well as recordings made in the same region from 1967-1971 and annotated by the Paynes. Winn et al. (1970) supported this discovery with a separate description of the patterned sequences of sounds recorded from humpback whales in the West Indies.

From that time on, investigations regarding the complexity, function, and pattern in humpback whale song have increasingly grown in number. In 1979, the first paper attempting to quantify signature information in song units was published (Hafner et al. 1979). The authors did not recognize the significance of the evolving nature of humpback song at this time, though this was soon to be described in detail (Payne et al. 1983, Guinee et al. 1983 Payne & Payne 1985). Singing humpback whales were shown to be males (Glockner 1983), and the function of song within the breeding season was hypothesized to play a role in female attraction (Winn & Winn 1978, Herman & Tavolga 1980, Tyack 1981) or male-male competition (Darling 1983). Further studies have compared song patterns across regions (e.g. Payne & Guinee 1983, Helweg et al 1990, Cerchio et al. 2001, Darling & Sousa-Lima 2005), or seasons (Winn & Winn 1978, Matilla *et al.* 1987, Noad et al. 2000, Eriksen *et al.* 2005), demonstrating three of the fundamental principles of humpback song: 1) males within an ocean basin sing similar songs, 2) features of songs differ between ocean basins but the overall hierarchical structure remains similar across regions, and 3) song structure evolves as a result of individual males changing both the spectral and temporal features of song units.

Between the 1970s and early 1980s, the terminology used to describe humpback whale songs was firmly cemented within the scientific literature (Payne & McVay 1971, Payne et al. 1983). Other authors occasionally varied this vocabulary (Winn & Winn 1978), but for the most part it has remained in use as originally proposed. This terminology is not, however, without complications, and inconsistencies in its application have led to incongruities in the literature.

There is a fair amount of variation in humpback whale song, both within the songs of an individual and between the songs of individuals (first noted by Payne & McVay 1971). Early on, Frumhoff (1983) conducted an extensive review of “anomalous” songs. And while early analyses suggested that song sequence was extremely stereotyped (Payne and McVay 1971; Winn and Winn 1978), more recent studies have demonstrated that song structure is not as consistent as was first thought (Helweg et al. 1990, Helweg et al. 1992, Helweg et al. 1998, Eriksen et al. 2005). Measurements of variation both on the overall structural level, as well as on the level of individual song units, are clearly important for understanding the function of song within this species and the influence of selection on singing behavior.

Consistent and well-defined criteria for the classification and measurement of humpback whale song features are essential for robust comparisons between investigators. Because we, as scientists, impose arbitrary divisions on what is otherwise a continuous vocal sequence, these divisions are sometimes constructed inconsistently. Additionally, in light of ongoing research, the vocabulary derived by Payne & McVay (1971) to describe the hierarchical structure of humpback song is in some cases incompatible with the observed song characteristics. The objectives of this review are to: 1) suggest consensus criteria and rules for song structure definitions and discrimination, and 2) engage in a discussion on the use of the term “song” in the humpback system, with some suggestions for revision.

These suggestions are offered to better enable comparable quantitative analyses between investigators. We are aware that our suggestions might not be suitable to all investigations/investigators. Thus, we urge future authors to explicitly describe the way they generate the humpback whale song metrics in their publications.

Hierarchical levels

Overview

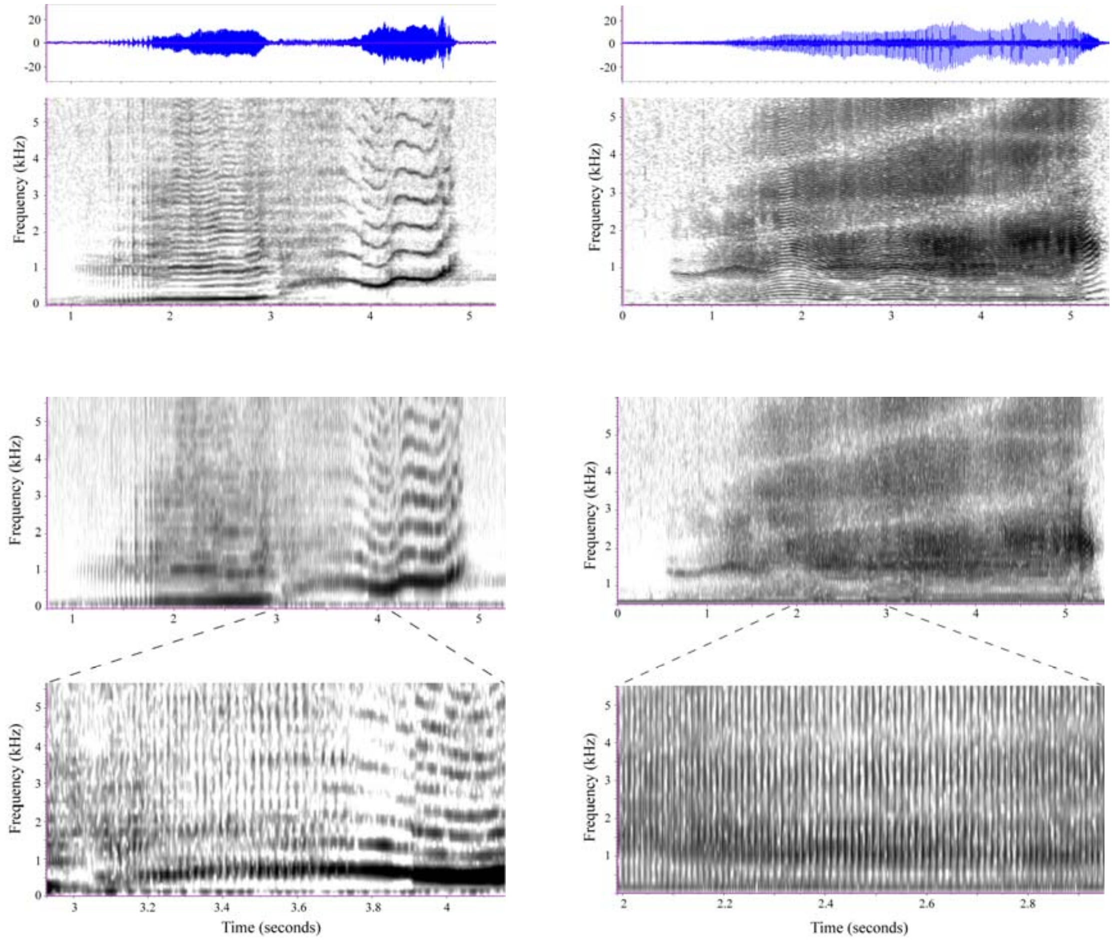
Payne & McVay (1971) presented both a descriptive and a graphical display of their suggested hierarchical levels in humpback whale song. The shortest sound is called a **unit**, which may be divided into **subunits** if comprised of pulses that are too rapid to be discriminated at real speed. A set of units is combined to form a **phrase**. Similar phrases are repeated to form a **theme.** The **song** is defined as the combination of several distinct themes. The highest hierarchical level is the **song session**, which consists of a series of repeated **songs** with silent intervals of less than a minute.

Subunits and Units

A **subunit** is a component of a sound that is perceived as continuous when heard in real time (Payne & McVay 1971). When examined with appropriate spectrogram parameters or played a slower rate, these seemingly continuous sounds can be shown to be composed of discrete pulses (Figure 1.1.1). Grating or rasping sounds are often composed of pulse-like subunits, but are considered a single sound nonetheless.

A **unit** is defined (Payne & McVay 1971) as the shortest sound that seems continuous when evaluated at real speed (Figure 1.1.1). This is analogous to a “note” in the avian song literature (Issac & Marler 1963). Winn & Winn (1978) called these individual sounds “syllables”, which is somewhat confusing, as “syllables” in the avian literature may actually be composed from groups of notes that have internote intervals of silence that are shorter than the duration of the adjacent notes (Isaac & Marler 1963). The term **unit** has been more widely used within the humpback literature, and should be used in place of the term syllable.

We suggest that the hierarchical levels of unit and subunit be maintained. These definitions are simple and unambiguous allowing robust comparisons.



i.

ii.

Figure 1.1. Two different examples of a humpback song unit containing subunits, recorded at Isla Socorro, México. The top panel in each column displays the amplitude envelope, and the lower panels display the spectrogram (Hann window, 50% overlap) or part thereof, showing the unit and subunit structure. i) Recorded 27 March 2006. This middle segment of this song unit contains a section composed of subunits. Top panel: 1024 pt FFT; middle panel: 256 pt FFT, bottom panel: 256 pt FFT. ii) Recorded 8 April 2004. Typical "ratchet" sound, in which the entire song unit is composed of rapidly produced pulses. Top panel: 1024 pt FFT; middle panel 256 pt FFT, bottom panel: 128 pt FFT.

Subphrases and Phrases

A s**ubphrase** (Payne et al. 1983) is a repetition of one or more units (Figure 1.2). These units of repetition were alternatively called “motifs” by Winn & Winn (1978), who divided them into two types: similar (containing only one type of “syllable”, or unit, repeated several times) or dissimilar (containing two or more different “syllables”, or units, which are repeated in combination several times).

Two or more subphrases are grouped into a **phrase** (Payne & McVay 1971)**.** A phrase may be considered the most consistent element of repetition within humpback song, and similar phrases are generally repeated several to many times before a different phrase type is introduced (Figure 1.2).

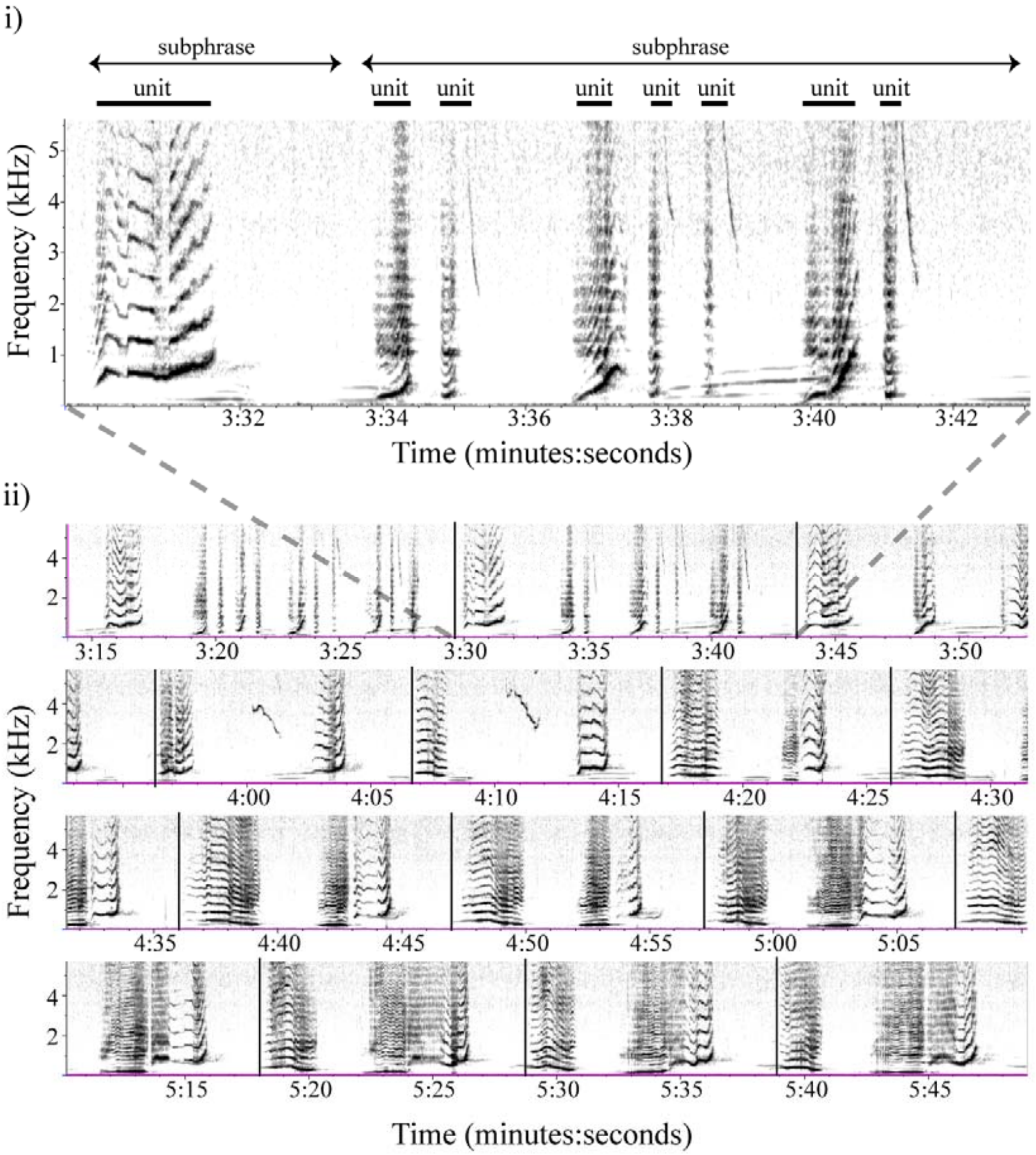


Figure 1.2. Spectrographic representation of humpback whale song sequence, recorded at Isla Socorro, México, on 27 March 2006 (1024 pt FFT, Hann window, 50% overlap). Time on the x-axis is in minutes:seconds, while frequency on the y-axis is in kHz. i) One phrase consisting of two subphrases. The first subphrase is composed of one unit, while the second subphrase is composed of a repeating pattern of 2-3 individual units. ii) 155-second sequence of song, in which multiple types of phrases can be observed. Phrases have been delineated by vertical lines.

Each rendition of a phrase may be sung with a high degree of consistency, or may be quite variable. This was documented by Payne & McVay (1971), who noted that phrases are "inexact replicas" of one another. Consecutive phrases may have different numbers of units, as well as a change in the spectral and/or temporal characteristics of the units, while still being identifiable as belonging to the same set of phrases. A phrase that combines some units of one phrase type and some units of another is called a "transitional phrase" (Payne et al. 1983). A transitional phrase is usually composed of an entire subphrase from the previous and subsequent phrase types (e.g. the phrase sequence: ab ab ab **ad** cd cd cd, where **ad** is a transitional phrase, composed of subphrase 'a' and subphrase 'd').

Unfortunately, the delineation of phrases may be difficult and ambiguous. In contrast to bird song, where inter-song intervals are noticeably longer than inter-note intervals (Isaac & Marler 1963), in humpback song the intervals between successive phrases may be the same as the intervals between units within a phrase. Additionally, although the element of repetition may be obvious upon examination, deciding which units should be used to begin or end a particular phrase may be unclear. Since this division is subjective, differences in methods between authors can make it challenging to compare studies, even of song within the same region. For example, two different authors analyzing song recorded off Hawai'i in 1979 divided the same sequence into 4 types of phrases (Thompson & Friedl 1982) and 7 types of phrases (McSweeney et al. 1989). Irregularities such as these often muddle the biological interpretation of the comparisons.

For clarity, we suggest the following guidelines for delineating and measuring phrases, which could be adopted in any song:

1) Consecutive units of similar structure should not be separated within a phrase, but should be kept together as parts of a subphrase.
2) If there is no distinct longer interval between units, phrases should be delineated in a way that minimizes the number of incomplete, or "hanging" phrases.
3) If there is ambiguity in unit position assignment within consecutive phrases (e.g., due to the presence of transitional phrases), then we urge authors to search for consistency in:
    i. Inter-individual pattern, or
    ii. Consistency in subphrase structure from one theme to the next.

   In the first case, if all recorded singers are singing a sequence that ends with an incomplete phrase, the 'extra' units can be assigned to the end of the previous phrase, thereby changing the unit composition of that phrase.
4) Duration of phrases should be measured including the interval between phrases (i.e., measuring from the onset of the first unit in one phrase to the onset of the analogous unit in the subsequent phrase). Measurements made in this way will be robust regardless of how one chooses to delineate phrases. In fact, if measured consistently, phrase duration has the least variation within and between singers of a particular population (Frumhoff 1983, Cerchio 2001).

We suggest that the hierarchical level of the phrase be maintained, with closer attention being given to criteria used to delineate phrases. When possible, a multi-year review of song should be conducted to examine phrase pattern and evolution, which may aid in classification of phrase organization. Additionally, review of song based on recordings of multiple individuals is essential for appropriately assigning phrase presence and structure.

Differing from Payne & McVay's original interpretation (1971), we also propose that the phrase hierarchical level is most analogous to a "song" in the avian literature (see below for further discussion on this topic).

Themes

A sequence of similar phrases is defined as a **theme** (Payne & McVay 1971**.** Individual males may sing different numbers of phrases in different themes and in consecutive renditions of the same theme. Thus, the length of any given theme varies both within and between individuals. In the past literature, Frumhoff (1983) classified **fundamental** themes as those present in all songs in at least 90% of the recordings in both a given season and at least one contiguous season. Other authors have classified "fundamental" themes as those present in 95% of all recordings within one season (Chu & Harcourt 1986). Due to the evolving nature of humpback song, the concept of a fundamental theme is ephemeral.

Payne & Payne (1985) further classified themes into three different types based on their organization: 1) **Static themes** are those with a sequence of nearly identical phrases; 2) **Shifting themes** are those in which successive phrases evolve progressively from one form to another. Units may gradually change in frequency and/or form, duration, or number of subunits, or be delivered at a slower or faster rate (i.e. variation in inter-unit interval). Therefore, the phrases may evolve such that changes are systematic and irreversible with each successive repetition; 3) **Unpatterned themes** are those in which a variable number of units have no clear organization and thus cannot be subdivided into repeating phrases. This results in a theme composed of one single, long phrase. Another type of one-phrase theme noted by Payne & McVay (1971) occurs when a single phrase is composed of unique material that does not resemble the previous or next theme but yet occurs consistently.

The classification of fundamental themes has not been widely adopted in the literature. The categorization of themes into types has also not been widespread, but our experience is that Payne & Payne's (1985) proposition of static, shifting, and unpatterned themes does characterize what we observe in humpback whale singing behavior in across many regions and years.

Care should be taken when assigning numbers or names to themes when comparing songs from different regions or time periods. Similar themes should be labeled as such, regardless of their position within the song sequence or the presence or absence of other themes. In one study (Maeda et al. 2000), what appear to be similar themes in two different regions are mistakenly numbered differently, due to presence of an extra theme in one of the regions. The analyses of theme presence and evolution are therefore incomparable between the two regions, complicating interpretation of the results.

We suggest that the definition of a theme be clarified to emphasize subphrase structure, such that a theme is a repetition of phrases that have similar subphrase types in common. Within-subphrase variation is acceptable such that the number of units between subphrases of the same theme may vary. Between two phrases, when one subphrase is similar but another subphrase is consistently different, a new theme should be designated based on the consistency of the structural change. Different morphs of the same phrase type that are sung inconsistently may be indicative of the process of evolution of a new "theme" (Payne et al. 1983). Therefore, when an entire subphrase changes in structure and the change is maintained throughout the song sequence, a new theme should be designated.

Song

A sequence of themes comprises a **song**, according to the definition proposed by Payne & McVay (1971). This description was further developed by Frumhoff (1983), who described a song as a series of at least three themes, organized in a predictable sequence, repeated in the same order two or more times. The choice of the theme that starts the "song" cycle is considered arbitrary, since males usually sing in a continuous bout without stopping between repeating cycles (e.g., in one famous example, Winn & Winn (1978) recorded a humpback whale singing for 22 h).

These and other early studies suggested that humpback sound patterns were produced in a fixed and ordered sequence (i.e., a male would sing themes 1 to 2 to 3 and then repeat the same sequence). This observation led to the conclusion that hierarchical structure existed at this level was that extremely stereotyped and rigid (Payne & McVay 1971, Winn & Winn 1978, Winn et al. 1981, Payne & Payne 1985, Guinee & Payne 1988).

It is undeniable that there is an overall cyclical pattern at this level of song organization (Figure 1.3). However, detailed reviews of the literature reveal that the stereotypy of theme order may vary considerably among and within individuals, or between years. Theme deletions were observed to occur coincidentally with the evolution of song, but reversals within the order of themes were rare (Payne et al. 1983, Payne & Payne 1985). Frumhoff (1983) coined the term "aberrant song session" as one in which a song cycle varied from the current norm; specifically, when two themes that are usually separated by a fundamental theme are sung in succession. In general, this was considered uncommon.

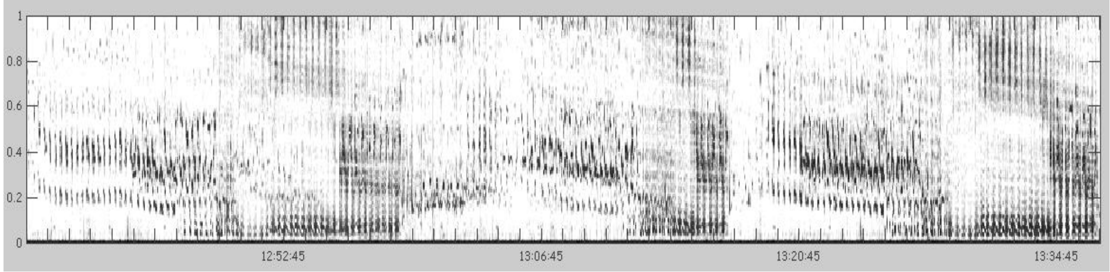
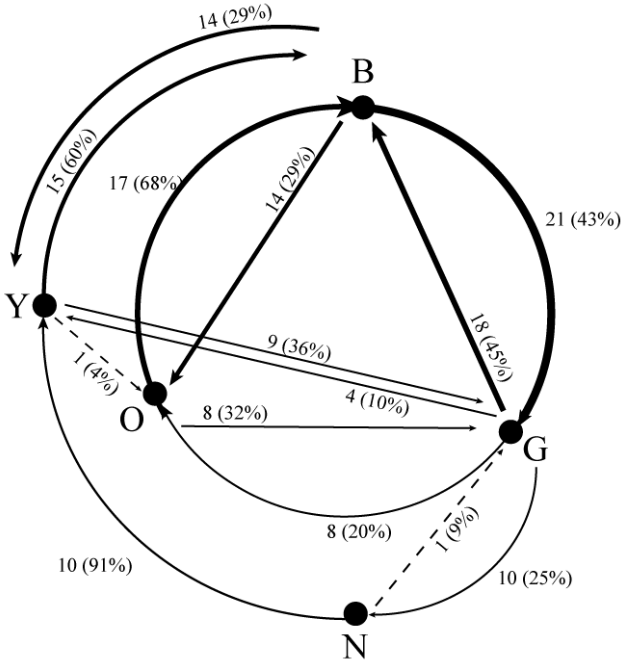


Figure 1.3. Spectrogram of 3500s of humpback whale singing activity recorded off the coast of Brazil in 2005 (FFT size = 256, Hann Window). The y-axis displays frequency in kHz, the x-axis diplays local military time. Note the cyclical pattern of the signal.

Later work, however, seemed to hint at a higher degree of variation in theme order. In a small sample of songs recorded in Mexico and Hawaii during 1989-1990, Helweg et al. (1990, 1992) recognized that the sequence of themes in the songs they recorded was variable (including some theme ‘reversals’), and suggested that all of their samples would be described as ‘aberrant’ by Frumhoff’s classification. In a separate study of South Pacific song (Helweg et al. 1998), theme deletions were observed, but theme reversals were not. However, in a multi-year comparison of song evolution in Tonga, Eriksen et al. (2005) reveal that the order of theme transitions is not consistent from year to year. Although they do not provide data to quantify the number of seemingly “aberrant” song cycles, they graphically demonstrate that the song sequence in particular years of their sample is more variable than others, including a higher degree of theme ‘reversals’ in some years as compared to others.

Further evidence for variation in sequence consistency?

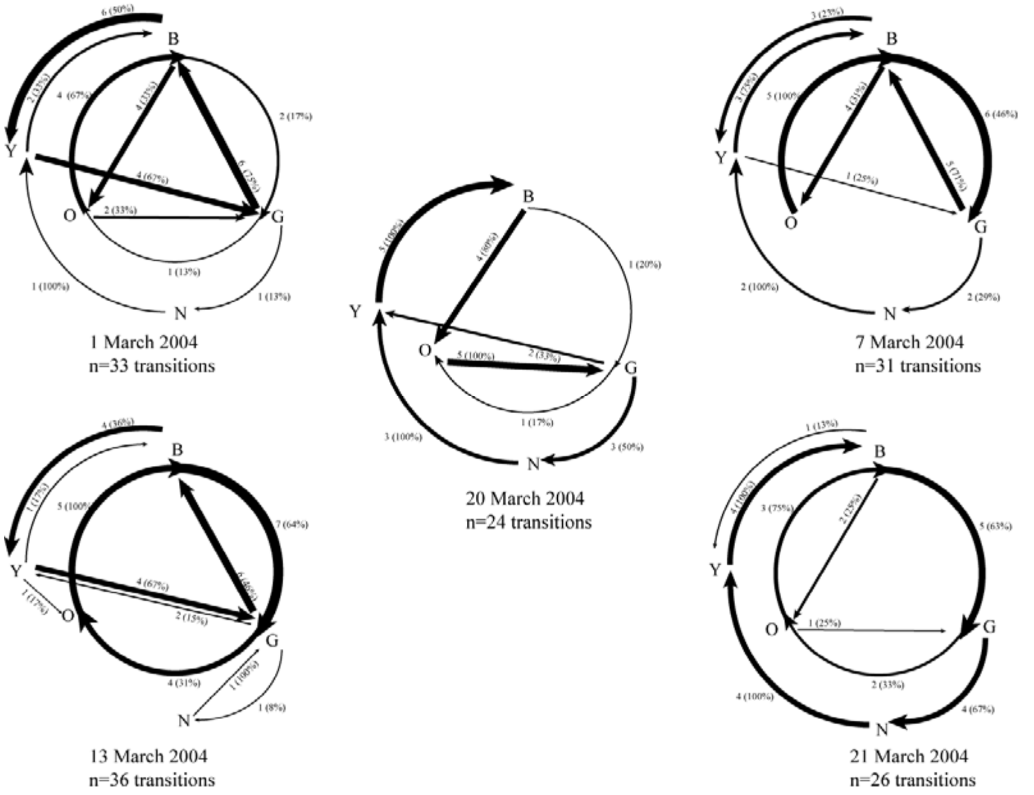
A multi-year study of humpback whale breeding behavior conducted off of Isla Socorro, México, has also resulted in the compilation of many hours of recordings of singing males across years. Recordings of males in 2004 suggest greater variability in theme order than typically expected. Rather than singing each theme in an ordered sequence, males recorded in this period appear to jump back and forth between themes, including a higher degree of "theme reversals" than previously reported (Figure 1.4).



n=150 Theme Transitions, 5 singers

Figure 1.4. Transition diagram displaying the numbers and percentages of transitions between pairs of themes. Continuous recordings of five males were combined for 5.8 hours of theme sequence analysis, resulting in 150 total theme transitions. Themes were designated by letters in this case, to avoid any unintentional bias associated with designating themes by sequential numbers. The numbers along each line indicate the number of total transitions from one theme to another; the percentages indicated the proportion of transitions from any one state to another state (i.e., there were 21 total transitions from theme B to theme G. This type of transition represented 43% of the transitions from theme B to any other theme The other 57% of transitions from theme B were represented by transitions to theme O or theme Y). Some theme transitions were extremely rare or non-existent, while others were prominent. However, a clear cyclical pattern is not observed, as reversals between themes B & G, O & B, and B & Y are common. Note that transitions between phrases within each theme are not represented here.

The observed overall variability in theme order is not due to one anomalous male, but is rather the result of multiple males each singing the same themes in varying orders (Figure 1.5).



1 March 2004
n=33 transitions

7 March 2004
n=31 transitions

20 March 2004
n=24 transitions

13 March 2004
n=36 transitions

21 March 2004
n=26 transitions

Figure 1.5. Transition diagram displaying the numbers and percentages of transitions between pairs of themes (see Figure 1.4 for detailed explanation). Each diagram represents the continuous recording session of one individual male. Recordings ranged from 50- 95 minutes. The number of total theme transitions for each male is given under each diagram. Note the lack of consistency between males in the transition probabilities.

In this situation, defining what constitutes a "song" by the classic definition is extremely difficult. There appears to be no clear, overarching sequence that all males are following while singing. This is not to say that the sequence of themes is random – in fact, examination of the transitions between themes reveals that some transitions are common (e.g., the transition from theme B->G, or theme O->B), while others are absent (e.g., there is never a transition from theme B->N). However, the difference in the cyclical pattern between the theme sequences of the males in this sample, and those singing a more traditionally ordered song, can be visually confirmed by comparing a spectrogram of the recording of one of these males (Figure 1.6) with the spectrogram in Figure 1.3 (where the singer's 'cyclical' pattern is visually obvious).

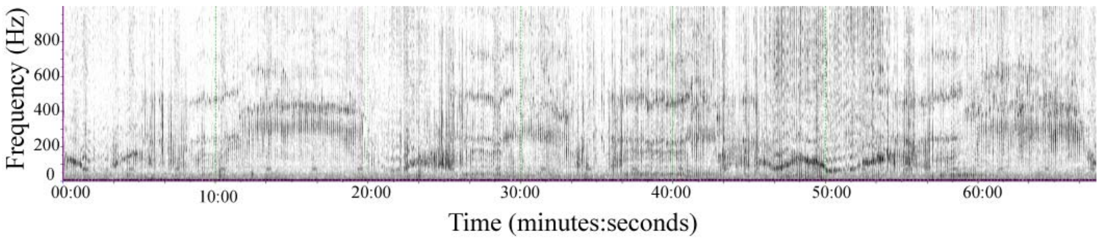


Figure 1.6. Spectrogram of 70 minutes of song recorded from one male on 7 March 2004 (256 pt FFT, Hann window, 50% overlap). The y-axis displays time in minutes and seconds, the y-axis displays frequency in Hz, up to 1000 Hz. The traditional "cyclical" pattern observed in humpback whale song is not observed in this example.

Humpbacks as "eventual variety" singers

Without more detailed study, it is impossible to say whether the variability in theme order observed in the songs of males recorded in Socorro 2004 is a short-term phenomenon or whether it is indicative of a larger-scale process. The only extensive, long-term study conducted on song evolution over many years (Payne & Payne 1985) found that occasionally the song in a particular year seemed anomalous when compared to previous and subsequent seasons. Possibly, the variability observed in the Socorro 2004 sample can be attributed to a period of rapid song evolution, or, possibly, it may reflect a broader cycle of the ebb and flow of stereotypy across years.

However, regardless of the underlying reason for this variability, its existence and the existence of variation in theme order in other samples (e.g., Eriksen et al. 2005) suggests that, in at least some cases, a different method of considering humpback whale song may be appropriate.

There does exist terminology in the avian literature that seems to effectively describe the humpback song system, and may be used in situations such as these. Scholars of avian behavior defined bird song as a series of notes, uttered in such a way as to form a recognizable sequence (Thorpe 1961; Bremond 1963). This definition also included the temporal pattern, in which the pauses between notes were shorter than the pauses between 'songs' (Isaac & Marler 1963; Thielcke 1969). Variations on these patterns were called 'song types'. Payne & McVay (1971) drew on this literature when developing their terminology, and equated a sequence of themes sung by a humpback whale with a "song" sung by a bird, but perhaps the term "song" would have been more appropriately applied to the level that Payne & McVay called a "phrase". If, for a moment, we consider humpback *phrases* and bird *songs* to be analogous, then we can consider each humpback *theme* as a different *song type*, and the compilation of themes presented by any one singer as his *repertoire*.

Early scholars of avian song noted different types of singing behavior, in which a bird may repeat the same song type many times, or switch immediately between different song types in his repertoire (Hartshorne 1956). The difference in these modes of singing was more fully developed in the literature to differentiate between "eventual variety" or "repeat" singers, and "immediate variety", or "serial" singers (Molles & Vehrencamp 1999).

Most studies to date have considered the humpback song sequence on the level that was originally proposed by Payne & McVay (1971), examining features such as "song duration", without giving thoughtful treatment to the variation in actual sequence on the phrase or theme level. Considering song in the “avian” framework would allow an analysis of phrase order over time without being hindered by how to define where the pattern begins or ends. Avian studies that address eventual variety songsters may include analyses of variables such as degree of repetitivity and switching rate - factors shown to be important in inter-individual interactions (Molles & Vehrencamp 1999; Molles 2006). By restricting ourselves to considering humpback “song” at only the broadest level, we have limited ourselves from similar types of analyses that may shed light on the perennial question regarding the function of humpback whale song.

“Song” as a problem

Besides limiting the scope of our analyses, why is the present definition of humpback “song” a problem? Consider the case in which the length or duration of a “song” is used as a quantitative metric to evaluate the impact of potential acoustic disturbance to singing whales (low-frequency active sonar experiments: Miller et al. 2000; Fristrup et al. 2003). Fristrup et al. (2003) used a very straightforward method to delineate sequential “songs”: a song was measured as the interval between successive starts of a particular theme (that which was traditionally associated with surfacing), without respect to theme order beyond this “marker” theme. The authors measured “song duration” ranging from 5.5 minutes to over 33 minutes in length, and conclude that while songs in general increased in duration in response to the acoustic disturbance, song length was highly variable. This is not surprising if the delineated “songs” actually encompass variable numbers of themes due to variation in theme order. Both this and another study using some of the same data (Miller et al. 2000) generally agreed in their conclusions, that singing humpbacks increased the length of their songs in relation to broadcasts of low-frequency active sonar.

But what of the observed high levels of variation in “song” duration? If theme order is fairly invariant (e.g., 123451234512345), then a measure of song duration is informative because each repetition through the cycle includes the same number of themes. We can then predict that longer songs may be achieved in one of several ways (i.e., by increasing the length of particular units or phrases, by increasing the number of phrase repetitions for the theme that was overlapped by sonar pings, or by increasing the number of phrase repetitions across the whole song).

However, if theme order is more variable, then consistently measuring “song” length becomes difficult. Consider the following example of an actual theme sequence recorded in Brazil 2002, when six themes were clearly identified:

…1234124546123465461234546121246…

We can arbitrarily set the beginning of the song to “Theme 1”. If we categorize this sample based on the requirement that a “song” includes a complete rendition of all themes, our sequence would be as follows:

…1234124546 / 12346546 / 1234546 / 121246…

If instead we used Frumhoff’s (1983) definition, our “song” might be categorized by the repetition of themes 1-2-3 (since this pattern is repeated more than twice in the song session), perhaps leading to the following:

…1234124546 / 12346546 / 1234546121246…

Or, following the precedent of the Fristrup et al. (2003) study, we could start a new song each time we encounter theme 1. Thus our song sequence would be as follows:

…1234 / 124546 / 12346546 / 1234546 / 12 / 1246…

It is evident that these various methods of dividing the theme sequence lead to different results in terms of the number of “songs” recorded, in the measured duration of each song, and in the thematic composition of each song. As a result, attempts to derive biologically relevant interpretations from analyses at the “song” level become difficult at best, and interpretation of the results is impossible without description of the variability of theme order.

In the case of the low-frequency sonar study, Miller et al. (2000) stated that there was little difference in the likelihood of aberrant theme transitions across exposure periods. However, they did not quantify the proportion, or types, of aberrant theme transitions. Neither of the studies (Miller et al. 2000; Fristrup et al. 2003) presented data on variability in thematic composition or order in the song sequences, nor attempted to quantify the underlying reasons for the large variation or observed increase in “song” duration. As mentioned previously, it is possible that a longer “song” is the result of singers increasing the number of repetitions of phrases within (a) theme(s). Alternatively, males might sing a more “erratic” song, in which they switch back and forth before completing a cycle (i.e., returning to the theme that was chosen as the “beginning”). Knowing what type of response is responsible for an increase in “song” duration, as well as knowing what constituted a typical “song”, would be informative in interpreting the mechanism of the singers’ reaction. Biologically, these differences suggest distinct responses to acoustic interference, which in one case leads to a more repetitive, less variable song presentation over time, and in the latter case, might lead to a more variable, less repetitive song presentation. Unfortunately, without further information on theme order, the authors of these studies do not offer the reader any information with which to interpret what their “longer songs” actually represent. Perhaps a measure of breathing interval in these studies would have provided further clues as to the potential biological and physiological impact of the acoustic disturbance events.

This real example demonstrates that term “song” as applied to humpback whales is in some cases problematic, and raises the question as to the biological interpretation of analyses conducted at this hierarchical level. This is not to say that the term “song”, as classically defined for humpback whales, is without significance. When theme order is largely invariant, then cleanly dividing sequences into song cycles for analyses may be relevant because the “songs” being compared are true repeats of the same pattern. And clearly, in at least some years, male humpback whales do seem to adhere to a hierarchical sequence at this level, which is likely to be biologically important. However, if and when individuals incorporate a larger degree of variation into their thematic sequence (as in the example from Socorro 2004, or Brazil 2002), then trying to force this variation into a structure that is defined by a ordered repetition is not appropriate. The outcome is a series of arbitrarily determined “songs”, none of which is like the other. From a biological perspective, the degree of variation in theme order may be informative, but it is difficult to deal with in the current classification scheme. Therefore, the potentially relevant differences in humpback singing behavior is generally ignored, rather than being explored in detail. This may regrettably be limiting our understanding of the use of song within the humpback mating system.

A final note on song designation: while breathing intervals have sometimes been used as a “convenient way to define a beginning and end” of a song (Winn et al. 1970; Payne & Payne 1985), our own observations as well as other studies (Winn & Winn 1978; Winn et al.1981), document individuals breathing during different themes, so the temptation to use breathing cycle as a measure of “song length” should be avoided.

CONCLUSIONS

Investigations of humpback whale song have spanned over 3 decades, yet there are still many unanswered questions regarding the degree of variation within and between individuals, the ways in which changes in song structure are transmitted over space and time, and the role of song within the breeding system, to name a few. Pioneering work in the 1970s and 1980s developed a framework in which to analyze and understand humpback song structure, which was later reinforced and expanded by further studies. Humpback whale song is a constantly changing phenomenon, which has captured the interest of many students of animal behavior, while also presenting challenges with respect to qualitative and quantitative analyses.

At the lower hierarchical levels within the humpback song "framework", traditional definitions have worked well to identify song elements for analyses. At the middle levels, however, delineation of "phrases" and "themes" has been complicated both by the lack of well-defined time intervals between repetitive sequences, as well as by the ever-changing nature of song features. At the highest hierarchical level within this framework, the application of the term "song" has, at times, obscured the degree of variation in theme order that exists between males. While males have been shown to often sing themes in a relatively fixed order, it appears that in some regions and time periods there is variation in the consistency with which individual males adhere to a strict sequence. In the majority of studies to date, the potential biological relevance of this variability has been overlooked, because it is difficult to quantify within the traditional "framework". In these situations, we suggest that the measure of "song" (as a fixed sequence of themes) be used with extreme caution. It may be more appropriate to discard analyses at this level, instead quantifying song structure at level of phrases or repetitions of phrases (themes). Some authors have moved in that direction (e.g., using Levenshtein distance to compare sequences: Eriksen et al. 2005; Tougaard & Eriksen 2006). If we expand our conceptual framework to learn from studies of song in other taxa, we can also consider humpback whales as "eventual variety" singers. Theme sequences can be measured over blocks of time using similarity or complexity indices in an effort to describe and quantify the observed variation, rather than ignore it. Further developments along these lines, using quantitative metrics to measure the degree of repetition or variation over time rather than among song cycles, may be useful in advancing our understanding of the complex nature of humpback whale vocal behavior.

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CHAPTER TWO

DIEL PATTERNS IN HUMPBACK WHALE (*Megaptera novaeangliae)* SINGING ACTIVITY OFF ISLA SOCORRO, MÉXICO

ABSTRACT

Vocal activity levels of many terrestrial species vary with diel light cycles. Recent studies have documented this pattern for a number of marine mammal species as well. However, previous studies of humpback whale singing behavior in the breeding areas provide equivocal evidence as to whether males of this species exhibit a diel pattern in vocal activity. The current study employed an array of four autonomous seafloor recorders to record continuously for 35 days in 2004, during the winter breeding season of humpback whales off Isla Socorro, México. The number of singers (from 0 to 4 or more) was quantified for the first 10 minutes of each hour for the entire recording period. Mean RMS sound pressure levels were also measured for the first 4 minutes of every half hour for 17 days (5 - 21 March) on two recording units. Data were then grouped into three light levels (Dark, Light, Twilight). We rejected the null hypothesis that the mean number of males singing during each light level is equal ($p<0.0001$), and found significantly higher numbers of males singing during Dark and Twilight than during Daylight. Similarly, the null hypothesis of equal mean RMS levels for each light level was rejected as well ($p=0.0308$), with significantly higher RMS levels during nighttime (Dark) hours. Mean RMS sound levels increased an average of 3.3 dB per additional singer. There was a significant decrease in the average number of singers per day over the 35 days, but the diel trend in singing

activity was evident both in the first and last week of the recording session. If humpback males sing to attract females, the higher levels of singing activity at night suggest that female assessment of singers may be taking place during this period. Efforts to determine the function of humpback song may best be directed at night, when male singing activity is highest.

INTRODUCTION

Diel patterns of vocal activity are common among many terrestrial taxa, with various species exhibiting peaks in activity in the evening or nighttime hours. For example, many anurans increase their calling rates during or after dusk, with some species reaching a peak in vocal activity well after midnight (Bridges & Dorcas 2000; Fritzsch et al. 1988). Various mammals are known to call more at night, including coyotes (Walsh & Ingles 1989) and primates (Drubbel 1993; Geissmann & Nijman 2006). Several notable species of songbird exhibit their highest rates of song activity at night, among them the common nightingale (Thomas 2002). Some otherwise diurnal songbirds may also sing during the night, though at lower rates than during the day, such as marsh wrens (Barclay et al. 1985). Additionally, the remarkable phenomenon of the “dawn chorus” is the result of many species exhibiting an intense peak in song output at or before daybreak (Staicer et al. 1996), and as the breeding season progresses, individual males may begin singing earlier and earlier before dawn (Staicer et al. 1996).

The factors influencing these patterns in vocal activity among groups are diverse, and may include social, physiological, and environmental variables. In most cases, patterns in vocal activity level are an epiphenomenona of the circadian rhythm governing an animal's general activity level, so those species that are more active at dusk or during crepuscular hours would be expected to have higher rates of vocal activity during those periods. In some cases, for example with many anurans, breeding behavior may be linked to environmental conditions (such as temperature), and increases in vocal activity may correspond to times of day when conditions are appropriate (Prestwich 1994). In other cases, however, there may be ultimate selective pressures leading to diel patterns in vocal activity. In the case of the avian dawn chorus, many hypotheses have been proposed to explain why individuals put so much effort into singing at this time of day. Broadly, these include maximizing communication probability when conditions for sound propagation are optimal, maximizing reproductive effort when foraging conditions are suboptimal, initiating social interactions which are best established at dawn, or responding to changes in hormone levels that follow circadian patterns (Staicer et al. 1996).

Research in recent years has uncovered diel trends in vocal activity among a variety of marine mammal species, including pinnipeds (Rouget et al. 2007; Van Parijs et al. 1999), sirenians (Ichikawa et al. 2006), delphinids (Goold 2000), and baleen whales (Au et al. 2000; Matthews et al. 2001; Stafford et al. 2005; Wiggins et al. 2005). The contexts in which these animals are communicating include foraging, territory defense, and breeding, so the selective pressures influencing vocal behavior differ markedly among species. For example, harbor seals have been shown to call more at night, but this may be strongly linked to tidal cycles and haul out patterns (Van Parijs et al. 1999). In winter, Weddell seals show an obvious increase in calling rates at night, which may be linked to the need to defend breathing holes in the ice in darkness (Rouget et al. 2007). Among delphinids, calling may be linked to foraging activity. If foraging activity is higher at night (perhaps due to movement or distribution of prey species), species that are cooperative foragers may be more vocally active during this period (Benoit-Bird & Au 2003).

Among baleen whales, diel trends in vocalizations have been reported for three species: blue whales, humpback whales and right whales. Recordings made of North Atlantic right whales in spring and summer (Matthews et al. 2001), suggest that “moans” and “gunshot” sounds may be more frequent at night than during the day, perhaps owing changes in social activity and aggregation size at night. Two recent studies with year-round monitoring for blue whales (Stafford et al. 2005; Wiggins et al. 2005) have found that blue whales produce at least one call type more frequently at twilight or night than during the day. These studies are particularly interesting in that they examine vocal activity levels during distinct and complementary periods in the blue whale annual cycle. Both in the summer and fall in the North Pacific, as well as during winter and spring in the Eastern Tropical Pacific, blue whales were found to produce B calls at higher rates during evening or nighttime hours than during the day. The function of this call is not well understood, though it occurs both as part of a song sequence as well as an isolated call, and only males are thought to produce it (McDonald et al. 2001; Oleson et al. 2007). Hypotheses to explain the observed diel pattern for this call type suggest a link between calling and foraging (Stafford et al. 2005; Wiggins et al. 2005).

Humpback whales migrate from high-latitude areas where they are feeding during the summer, to low-latitude breeding areas where they are fasting and calving during the winter (Dawbin 1966). The main vocal display during the breeding season is a long, relatively complex song (Payne & McVay 1971), produced only by males, although males also spend time engaged in other reproductive tactics and other social vocalizations are occasionally heard. Three different studies have investigated diel patterns in singing activity on the breeding grounds, with equivocal results. In 1987-

1988, Helweg and Herman (1994) collected acoustic data from a bottom-mounted hydrophone and quantified the number of singers heard and the signal-to-noise ratio measured every 2 hours for over 3 months. While they found that signal-to-noise ratio varied, they found no difference in the estimated number of singers at different times of the day and concluded that males sing continually. In a later study, Au et al. (2000) recorded for four minutes every half hour over an 85-day period from a single hydrophone mounted off the coast of Maui. The authors measured the daily root mean square (RMS) sound pressure levels (SPL) and evaluated two discrete periods (7-12 January and 5-21 March 1998) for evidence of diel differences in measured SPL. During the March period, the authors found significant differences in the SPLs for the 06:00h and 08:00h samples, and for the 17:00h and 1900h samples (local HI time). The average SPL recorded at night was several decibels higher than that recorded during the day. They concluded that the high SPL levels recorded at night during this period were linked to humpback breeding activity, and could be the result of one of several factors: an increase in the number of singers in the area, a movement of singers closer to shore (and closer to the hydrophone), or an increase in intensity of song output by individual singers. Finally, in a third study conducted off of Isla Socorro, México (Jacobsen & Cerchio 2001), five bottom-mounted recorders were placed in different locations around the island. The units recorded for 30s every 90 minutes over a 68-day period. The authors found differences in overall numbers of singers among the different areas, and also reported higher numbers of singers at night than during the day.

For humpback whales in particular, it is not entirely clear whether one would expect to see a diel trend in singing activity. Unlike delphinids, or blue or fin whales (Croll et al. 2002), humpback whales are not foraging during the season in which males are most often singing. Reproductive behaviors are separated both spatially as well as temporally from foraging behaviors, so there is no reason to predict changes in vocal activity with respect to movements of prey. Additionally, because sleep patterns in cetaceans are so different from those of terrestrial mammals (Ridgway 2002; Zepelin 1989), we do not necessarily predict vocal activity levels to change according to a typical terrestrial mammalian circadian rhythm. However, there may still be several scenarios under which we might predict singing activity to vary on a diel cycle: 1) if time spent engaged in other breeding activities (such as aggressive competition) varies depending on vision (and therefore light levels) or other sensory modalities, 2) if song detection area varies on a diel cycle as a result of changes in sound propagation or ambient noise levels, such that singing more at night is more effective, or 3) if female receptivity or the opportunities to attract a mate varies on a diel cycle. The goal of this study is to test whether a diel pattern in singing activity exists in humpback whales, and if so, whether it is dependent on the density of vocally active individuals, and quantify measured sound pressure levels with respect to numbers of singing individuals.

METHODS

Study Site

Research was conducted off Isla Socorro, México, one of several main breeding areas for humpback whales in the North Pacific (Urban et al. 1999; 2000). Isla Socorro is a small (~16 km in diameter), volcanic island, located within the Revillagigedo Archipelago at approximately 18º45’N 111º0’W (Figure 2.1). Humpback whales migrate annually to this breeding site and are found there from approximately December through April. Long-term research on humpback whale breeding biology has been conducted at this site since 1987, and the current study was conducted in conjunction with other simultaneous research.

Acoustic Data Collection

Acoustic data were collected using autonomous recording units ("pop-ups"), developed by the Bioacoustics Research Program, Cornell University (Clark & Clapham 2004; *www.birds.cornell.edu/brp*). Four units were deployed in a zigzag formation along a 6 km stretch of coastline on the southwest side of the island with an average separation of 1.7 km (Figure 2.1). Seafloor depth at pop-up locations ranged from approximately 35-100m. Three of the units recorded at a sampling rate of 2 kHz and were deployed from 2 March - 5 April 2004. The fourth unit recorded at a sampling rate of 10 kHz, and was deployed from 2 - 17 March, at which time it was retrieved and replaced with a new unit that recorded at 10 kHz from 17 March – 5 April. All units recorded continuously, 24h/day over the entire period (n=35 days).

Acoustic Data Analyses

Upon retrieval of the units, data from the 10 kHz recorders were filtered and decimated to 2 kHz, and data from all units were synchronized and merged to yield a total of 840 hours of 4-channel recordings.

Analyses were conducted using the software package Raven (Charif et al. 2007) and were divided into two parts. First, singing activity was measured by counting the total number of singers in the 10-minute period at the beginning of every hour, from 00:00h on 2 March through 23:00h on 5 April (n=840 10-minute sample periods). To do this, continuous spectrograms were generated for the entire dataset (512 pt FFT, Hamming window, 50% overlap) and were reviewed both visually and aurally. Singers were distinguished based on both time-of-arrival differences of song notes on different channels and by song patterns (Figure 2.2). A singer was counted as present if a song pattern could be visually identified on at least one of the four channels (non-song vocalizations were distinguished from song by their lack of repetitive structure; instances of non-song vocalizations were not included). A maximum of four simultaneous singers could be differentiated reliably across the channels. Therefore, singer counts ranged from 0 to 4 or more (hereafter referred to as 4+) and the maximum number of singers is likely to be an underestimate.

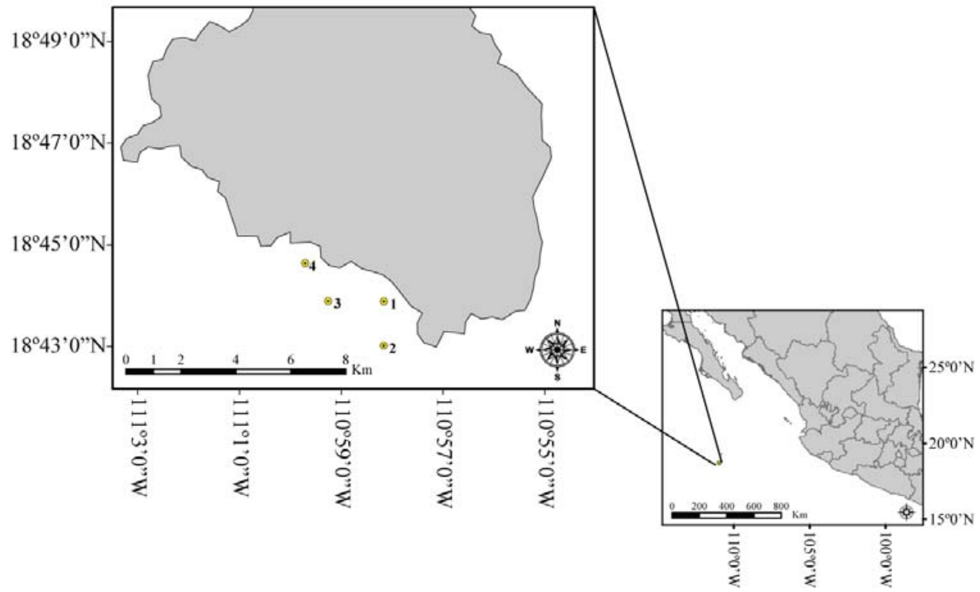
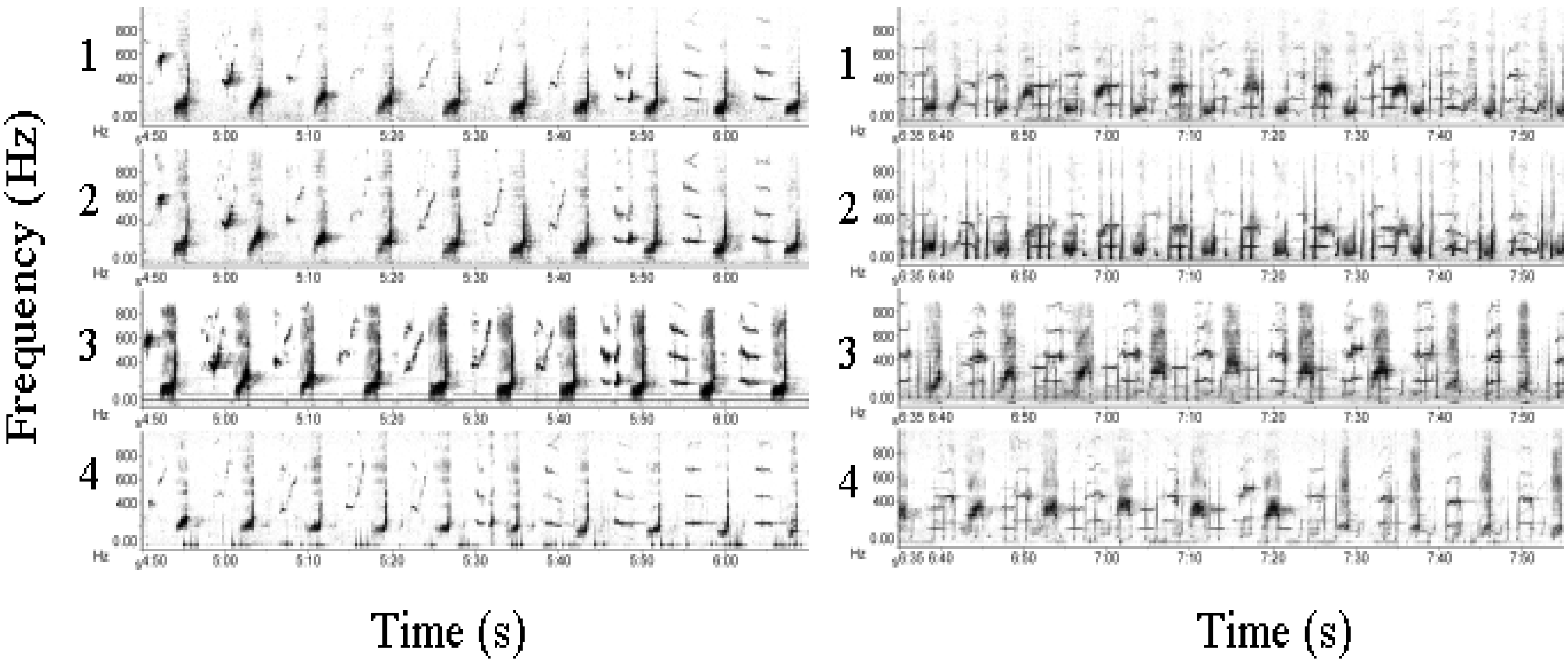


Figure 2.1. Isla Socorro, of the Revillagigedo Archipelago, México. Locations of the four autonomous recording units (pop-ups) are indicated by hexagonal dots.

Figure 2.2. 80-second samples of multi-channel spectrograms (512 pt FFT, Hamming window**,** 50% overlap) used to quantify the number of singers in each sample period. Each line in the spectrogram represents a simultaneous recording from one of four pop-ups (top line = channel 1 = Location 1, etc). The x-axis displays time in minutes:seconds, the y-axis displays frequency in Hz (from 0-1000 Hz for each channel). i) 7 March 2004 at 10:00h. In this example, only one singer is present on all four channels. ii) 5 March 2004 at 20:00h. In this example, four singers are present, distinguishable both by song pattern and time-of-arrival of song units on the different channels.


Frequency (Hz)
1
2
3
4
1
2
3
4
800
600
400
0.00
Hz
4:50
5:00
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5:20
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5:40
5:50
6:00
6:35
6:40
6:50
7:00
7:10
7:20
7:30
7:40
7:50
Time (s)
Time (s)

For the second analysis, root-mean-square (RMS) sound pressure levels (SPL) were measured separately for a subset of data from each of two pop-ups (Locations 1 and 2, Figure 2.1). To eliminate the effects of low-frequency noise and thereby more accurately test for changes in SPL due only to humpback whale song, data were highpass filtered at 36 Hz (FIR filter, -3dB at 31.43 Hz). The average RMS SPL was calculated from the time-waveform for the entire bandwidth of 36-1000 Hz for the first four minutes of every half hour, from 5 - 21 March (n=17 days). This analysis period was chosen so as to provide SPL data comparable with previously published results (Au et al. 2000). This sampling scheme resulted in a total of 816 four-minute sampling periods per pop-up.

To quantify background noise levels when no whale song was present, average RMS levels across the whole frequency band (36 – 1000 Hz) were quantified for 30 four-minute samples in which singers were absent.

Light Level Definitions

To evaluate singing activity as a function of a diel (day/night) cycle, three light levels were defined based on the geometrical angle of the sun relative to the earth's horizon: **Twilight** (defined for both pre-sunrise and post-sunset, where morning twilight is the period of time between the moment at which the center of the sun's disk is 12° below the horizon until the moment when the upper edge of the disk is on the horizon, and evening twilight is defined as the moment when the upper edge of the sun's disk is on the horizon until the moment at which the center of the disk reaches 12° below the horizon); **Daylight** (defined by the time between sunrise and sunset, when the upper edge of the sun's disk is on the horizon); and **Dark** (defined as the time between the end of evening twilight and the beginning of morning twilight).

Light-level definitions and corresponding time periods were calculated according to the U.S. Naval Observatory Astronomical Applications website (*aa.usno.navy.mil*) for 18°45’N and 111°0’W. Twilight levels were defined according to nautical twilight.

The times of day included in each light level were evaluated separately for the two different acoustic analyses, since they incorporated different numbers of days (35 vs. 17 days) and different time resolutions (hourly vs. half-hourly). All times are reported in local time (+7 GMT). For the analysis quantifying numbers of singers, samples analyzed between 20:00h-5:00h were categorized as **Dark,** samples analyzed between 7:00-18:00h were categorized as **Daylight**, and samples analyzed at 6:00h and 19:00h were categorized as **Twilight**. For the RMS SPL analyses, samples between 19:30-5:30h were categorized as **Dark**, samples between 7:00-18:00h were categorized as **Daylight**, and samples analyzed at 6:00, 6:30, 18:30, and 19:00 were categorized as **Twilight**. Morning and evening twilight were grouped into one category. Although there was an increase in day length of approximately 37 minutes over the 35 days, this did not affect the light categories used for this analysis, therefore, the times included within each light level were averaged over the analysis periods

Statistical Analyses

Statistical analyses were performed using the software package JMP 7.0 (©2007 SAS Institute, Inc.). The null hypothesis that the mean number of singers was equal for each of the three light levels (Dark, Daylight, Twilight) was tested using an ANOVA, posterior multiple comparisons were conducted using Tukey HSD. The null hypothesis that the mean RMS SPL level for each of three light levels was equal was tested using a non-parametric (Kruskal-Wallis) ANOVA. Pair-wise Wilcoxon rank-sums comparisons were subsequently performed (with Bonferonni corrected α-levels) to determine which light levels were significantly different from one another.

The null hypothesis that the mean number of singers per day was constant over the analysis period was tested using linear regression. The null hypotheses that the mean number of singers was equal for each of the three light levels was tested for a period of higher singer density (Week 1) and lower singer density (Week 5) using non-parametric ANOVAs. Posterior pair-wise Wilcoxon rank-sums analyses (with Bonferonni corrected α-levels) were subsequently performed.

Finally, change in mean RMS SPLs with respect to number of singers was tested using linear regression for the subset of RMS data for which singers counts were available on the same pop-up (n=137 samples).

RESULTS

Overall Patterns in Mean Numbers of Singers and Mean RMS Levels

Singers were detected on each day of the analysis period. The number of singers counted across the array for each hourly 10-minute sample ranged from 0 to 4+. When averaged by hour over the 35 days, singing activity peaked at 03:00h (2.7 ± 0.96 singers), and was lowest at 09:00h (1.3 ± 0.98 singers). A diel pattern in singing activity is clearly evident (Figure 2.3), with the lowest levels of singing activity during daylight hours, the highest levels of singing during nighttime (dark) hours, and intermediate levels of singing during morning twilight and evening twilight, respectively.

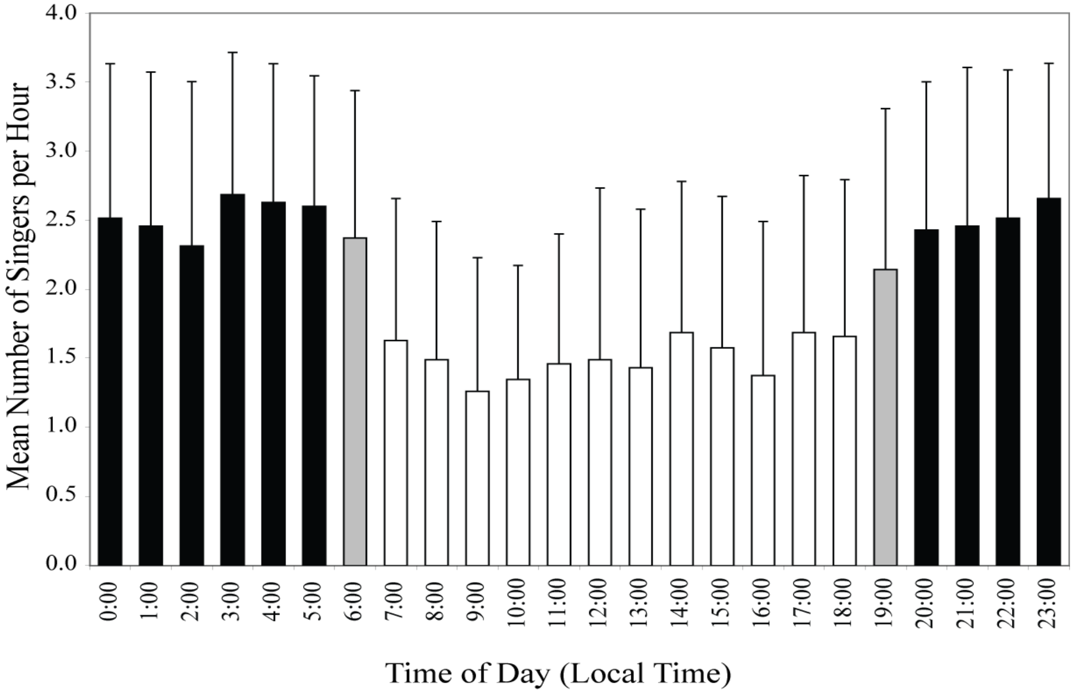


Figure 2.3. Mean number of singers per hour + SD, averaged over the 35-day analysis period (2 March – 5 April 2004, n=35 each hour). Number of singers counted in each hourly, 10-minute sample ranged from 0 to 4+, with 4 singers being the maximum number that could be reliably differentiated simultaneously across the four pop-ups. Bar color indicates light level: black (Dark), white (Daylight), and gray (Twilight).

Measured RMS sound pressure levels demonstrated the same general pattern as the counts of singers. Mean RMS levels ranged from 103-116 dB (re. 1 μPa) for the inshore pop-up (Location 1, Figure 2.4) and from 102-113 dB for the offshore pop-up (Location 2). A diel pattern in RMS levels was evident in both pop-ups, with higher SPLs during nighttime hours (peaking before morning twilight), lower SPLs during daylight hours, and intermediate levels during twilight hours. A peak in SPL was measured at 05:00 at both pop-ups, and SPL levels were lowest at 16:00h at both pop-ups. Further presentation of data will focus on the pop-up at Location 1.

RMS levels were not normally distributed. Data from the pop-up at Location 1 are bi-modal, with ~17% of samples measured with RMS levels of 95 dB or lower (Figure 2.5). These low RMS levels are in the range that corresponds to background ambient noise. Analysis of 30 samples in which singers were absent resulted in measured RMS levels of 93.7 ± 1.72 dB for the 36-1000 Hz frequency band. This is notably lower than the mean RMS levels for samples in which singers were present (110.3 ± 9 dB, n=376).

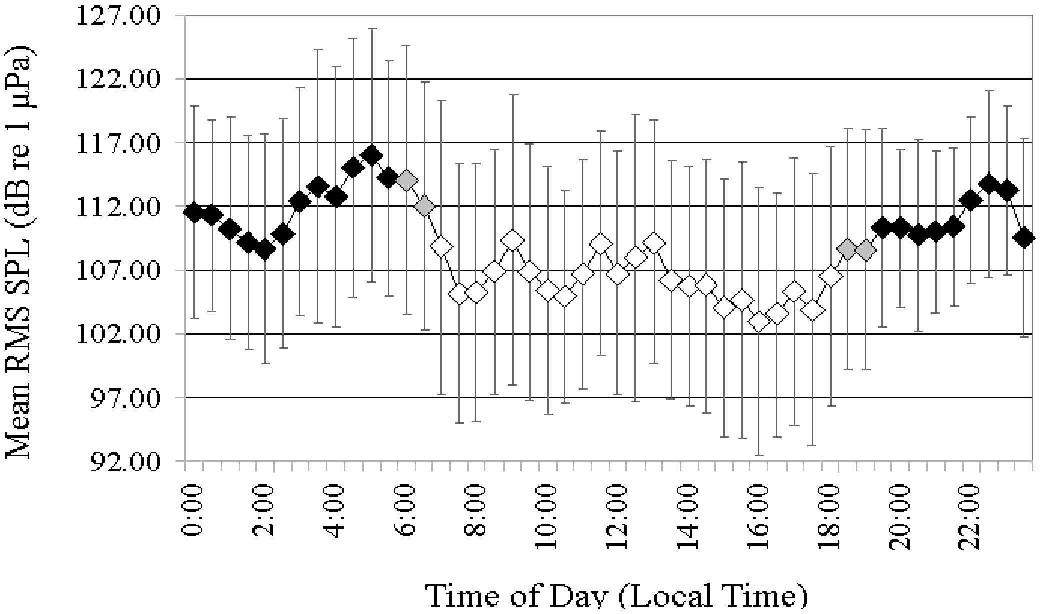


Figure 2.4. Mean root-mean-square (RMS) sound pressure levels (SPL) ± SD (dB re. 1 µPa) measured from pop-up at Location 1. One sample was taken for the first four minutes of every half hour, from 2 – 21 March (n=17 for each half hour). Data were highpass filtered at 36 Hz, and the RMS SPLs were calculated for the entire bandwidth (30 – 1000 Hz); black (Dark), white (Daylight), and gray (Twilight). Highest mean RMS levels at 116.0 ± 9.87 dB at 5:00 and lowest mean RMS levels were 106.5 ± 10.2 dB at 16:00h.

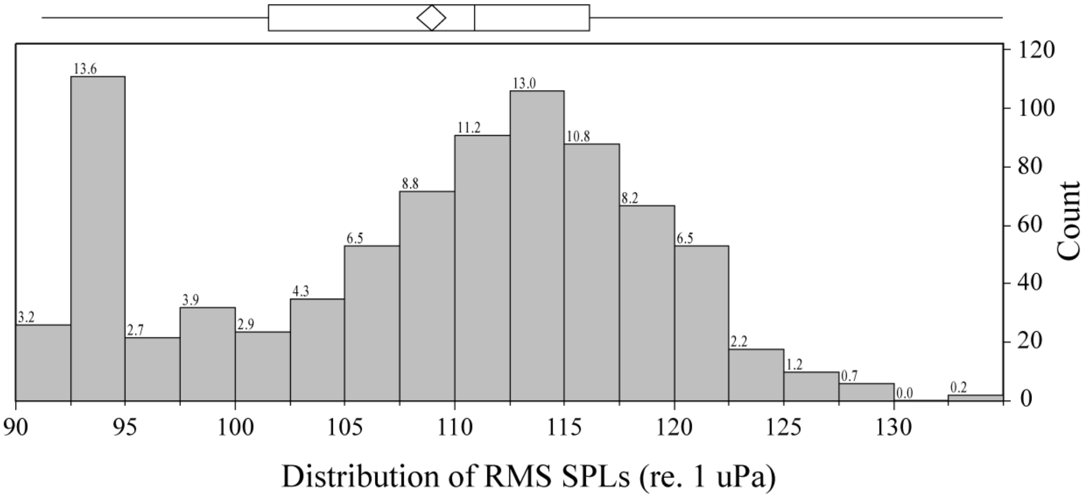


Figure 2.5. Distribution of RMS sound levels for all 4-minute samples (n=816) measured for the pop-up at Location 1. RMS levels ranged from 91.2 to 135.0 dB (re. 1 μPa). Each histogram bar represents 2.5 dB. Numbers above each bar indicate percentage of entire sample included within that the bar. Count data are indicated along the y-axis. Samples within levels of 93.7 ± 1.72 dB fall into the range of ambient noise.

Diel Analyses

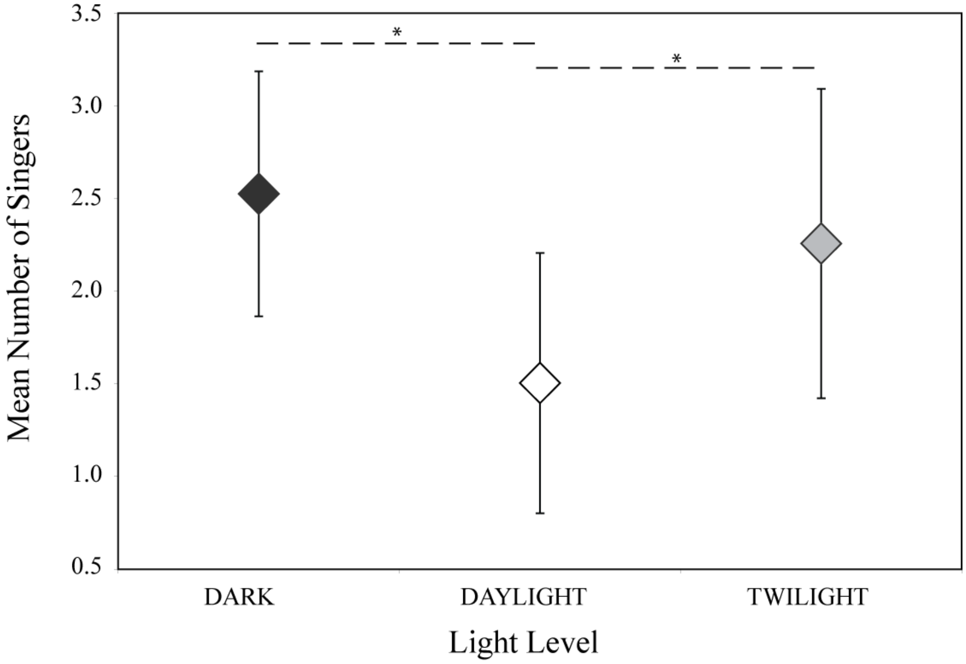
Mean numbers of singers were averaged per light level for each day (Table 2.1). The null hypothesis that mean numbers of singers were the same for each of the three light levels is rejected (ANOVA, F=18.0201, $p < 0.0001$, Figure 2.6). A Tukey HSD multiple comparison test was conducted to compare the mean number of singers for each pair of light levels, and the null hypothesis of equals means is rejected at the 0.05 α-level for Daylight vs. Dark and Daylight vs. Twilight, but not for Dark vs. Twilight.

Similarly, the null hypothesis that average RMS levels were equal for each light level is rejected (Kruskal –Wallis ANOVA, X^2 = 6.96, p=0.0308). Pairwise Wilcoxon tests rejected the null hypothesis of equal means for Daylight vs. Dark (p=0.0103), but not for Daylight vs. Twilight (p=0.0516), or Dark vs. Twilight (p=0.8497) (Figure 2.6).

Table 2.1. Mean number of singers and mean RMS SPL ± SD for each light level. A mean number of singers was calculated for each light level per day; these values were then averaged over all days (n = 35). Similar calculations were performed for the first week of recording (n=7) and the last week of recording (n=7). Similarly, the mean RMS level recorded was averaged for each light level per day for the pop-up at Location 1; these values were then averaged over all days (n=17).

	DARK (20:00-05:00h)	**DAYLIGHT** (07:00-18:00h)	**TWILIGHT** (6:00h & 19:00h)
Mean Number of Singers	2.5 ± 0.66	1.5 ± 0.70	2.3 ± 0.83
Mean RMS SPL (in dB)	111.6 ± 5.21	106.1 ± 5.79	110.9 ± 7.67
Mean Number of Singers: Week 1 (March 2 - March 8)	3.3 ± 0.34	2.3 ± 0.58	3.1 ± 0.54
Mean Number of Singers: Week 5 (March 30 - April 5)	2.1 ± 0.44	0.95± 0.49	1.4 ± 0.63

Figure 2.6. For both graphs, dotted lines with asterisks located above the standard deviation bars indicate light-level significance tests. **i)** Mean number of singers ± SD per light level (n=35 days). Means were 2.5 ± 0.67 (Dark), 1.5 ± 0.70 (Daylight), and 2.3 ± 0.83 (Twilight). Mean number of singers was significantly lower during Daylight as compared to both Dark and Twilight. **ii)** Quantile boxplots of average RMS sound pressure levels (re. 1 μPa) per light level (n=17 days). Mean RMS values for each light level were 111.6 ± 5.21 dB (Dark), 106.1 ± 5.79 dB (Daylight), and 110.9 ± 7.67 dB (Twilight). RMS levels were significantly lower during Daylight as compared to Dark.


3.5
3.0
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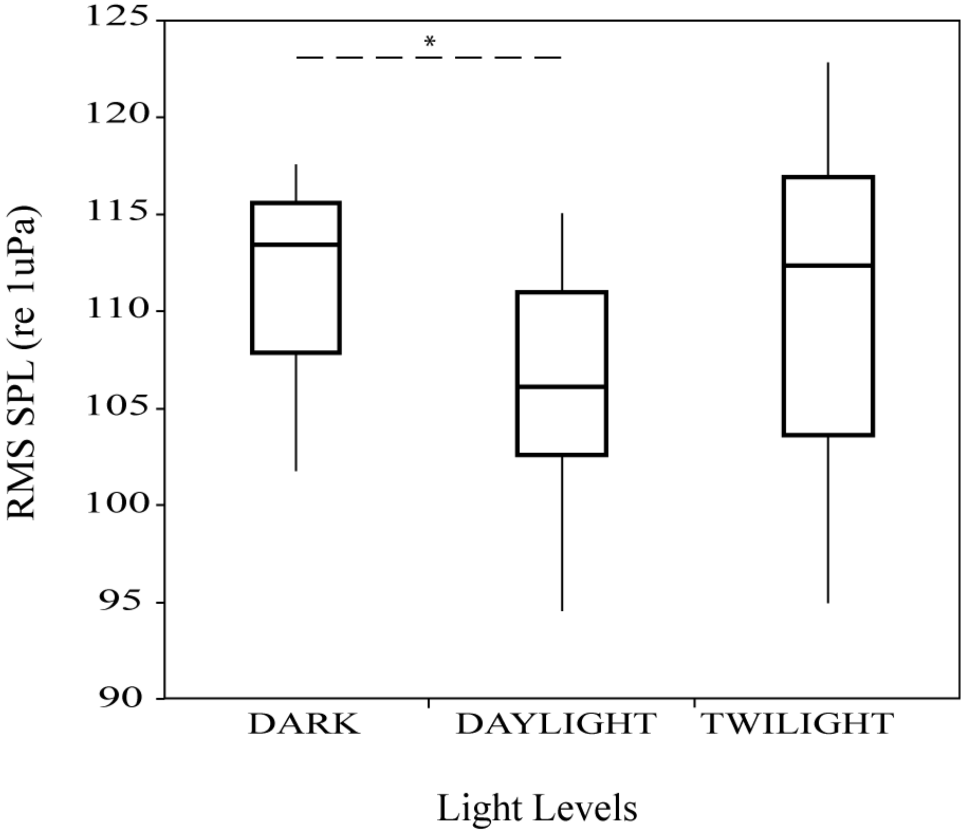
Mean Number of Singers
*
*
DARK
DAYLIGHT
TWILIGHT
Light Level

i)


125
120
115
110
105
100
95
90
RMS SPL (re 1uPa)
*
DARK
DAYLIGHT
TWILIGHT
Light Levels

ii)

Seasonal Trends

The average number of singers detected per day decreased significantly over the course of the analysis period (linear regression: $R^2 = 0.64$, $p < 0.0001$, Figure 2.7), with 3.3 ± 0.16 (SE) singers/day being the highest daily average (4 March 2004) and 0.96 ± 0.14 (SE) singers/day being the lowest (4 April 2004).

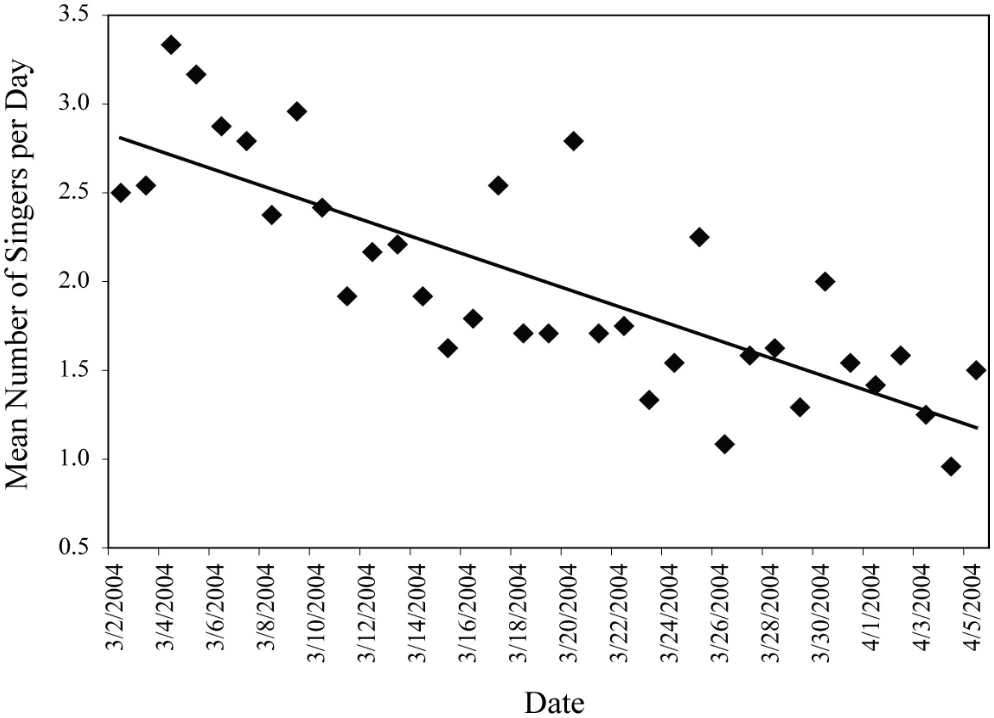
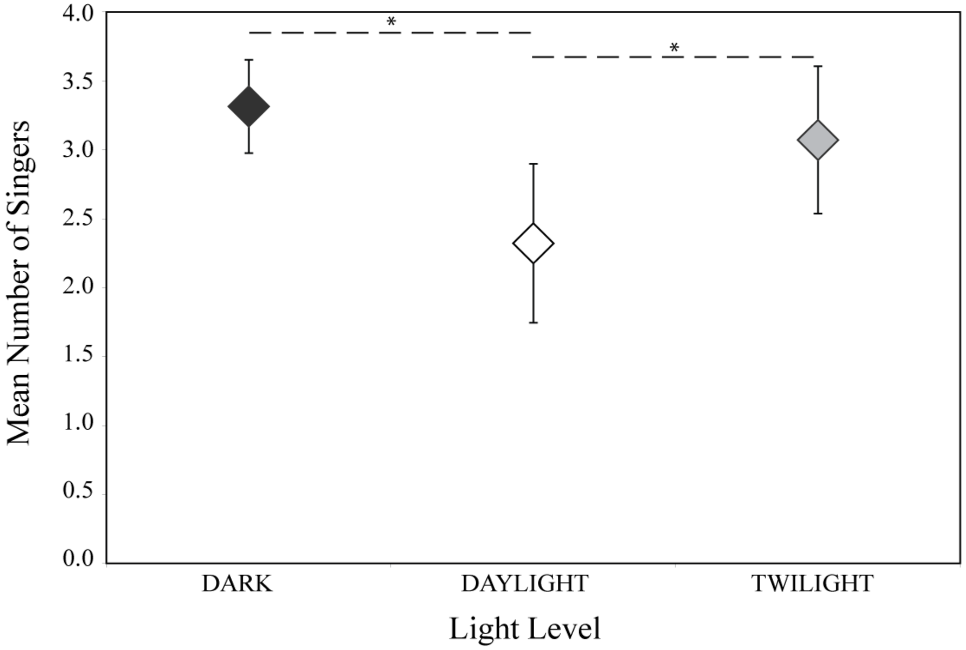


Figure 2.7. Plot of the mean number of singers per day over the 35-day recording period, from March 2- April 5, 2004. Mean number of singers significantly decreased over this period (linear regression: $R^2 = 0.64$, $p < 0.0001$)

We investigated whether the diel pattern in singing activity depended on average singer density by repeating the light-level analyses for the first week of the recording period (Week 1: 2 – 8 March 2004) and for the last week of the recording period (Week 5: 30 March – 5 April 2004) separately. The mean number of singers for each light level is reported in Table 2.1. The null hypothesis that the mean number of singers was the same during each light regime was rejected in both weeks (Kruskal-Wallis ANOVA, week 1: $X^2 = 8.6409$, $p = 0.0133$; week 5: $X^2=9.5338$, $p= 0.0085$; Figure 2.8), indicating that the diel pattern in number of active singers is not density dependent.

Pair-wise Wilcoxon tests for Week 1 revealed the same pattern as the overall dataset; the null hypothesis of equal means was rejected for Dark vs. Light (p=0.0086) and marginally so for Light vs. Twilight (p=0.0200), but not for Dark vs. Twilight (p=0.5154). In week 5, the overall mean number of singers per light level was lower. The null hypothesis of equal means was rejected for Dark vs. Light (p=0.0040), but was not rejected for the Light vs. Twilight periods (p=0.2721) or Dark vs. Twilight (p=0.0330).

Figure 2.8. i) Early week: Mean number of singers ± SD per light period for first week of recording period, 2 – 8 March 2004 (n= 7 days). Mean number of singers during Daylight period was significantly lower than Dark (p=0.0086) and Twilight (p=0.0200), but Dark and Twilight were not significantly different (p=0.5154). ii) Late week: Mean number of singers ± SD per light period for last week of recording period, 30 March – 5 April, 2004 (n=7 days). Mean number of singers was significantly higher during Dark period compared to Daylight (p=0.0040), while there was no difference in the mean number of singers in Daylight vs. Twilight (p=0.2721). Mean number of singers for Dark vs. Twilight (p=0.0330) was not significant at the Bonferonni corrected level.


4.0
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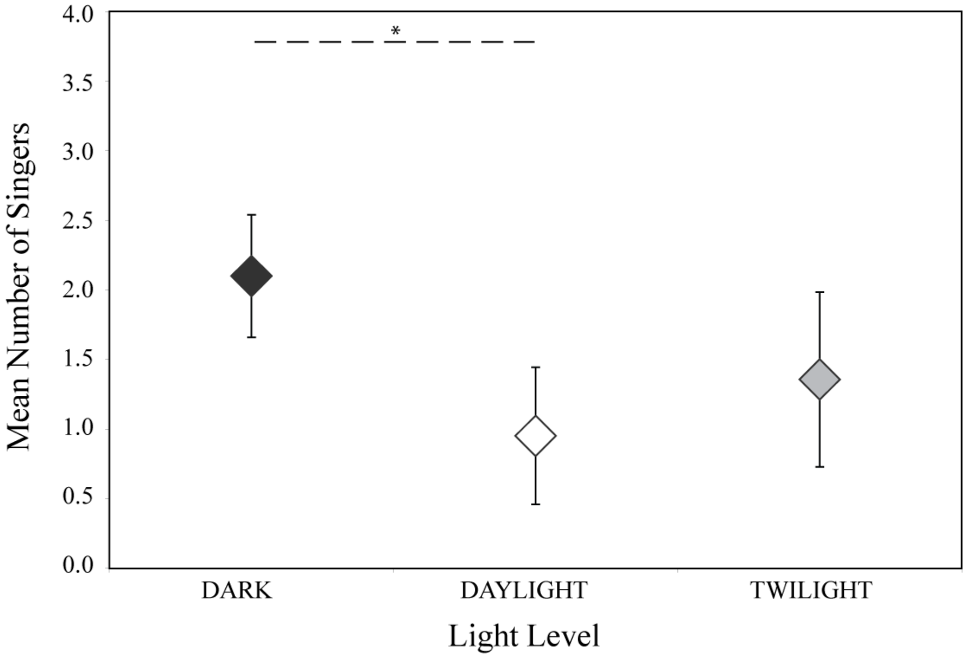
Mean Number of Singers
*
*
DARK
DAYLIGHT
TWILIGHT
Light Level

i)


4.0
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Mean Number of Singers
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DARK
DAYLIGHT
TWILIGHT
Light Level

ii)

RMS Levels by Singer

Finally, mean RMS levels per number of singers was calculated for a subset of data in which the number of singers were quantified for the same samples in which RMS levels were measured on pop-up at Location 1 (excluding samples with no singers, n=136 samples, Table 2.2). As expected, mean RMS levels increased as the number of singers present increased, with an average increase of 3.3 dB per additional singer (Figure 2.9). The variance in measured RMS levels was greater for samples in which only one or two singers were present, as in these cases the only singer(s) detected may have been quite close or quite far from the hydrophone. However, as the number of singers increased, the variance in RMS levels decreased, indicating that singers "packed into the area", so the overall distance between the hydrophone and the nearest singer was likely smaller and more consistent. The increase in RMS levels as the number of singers increased was significant (linear regression: $R^2 = 0.27$, $p<0.0001$).

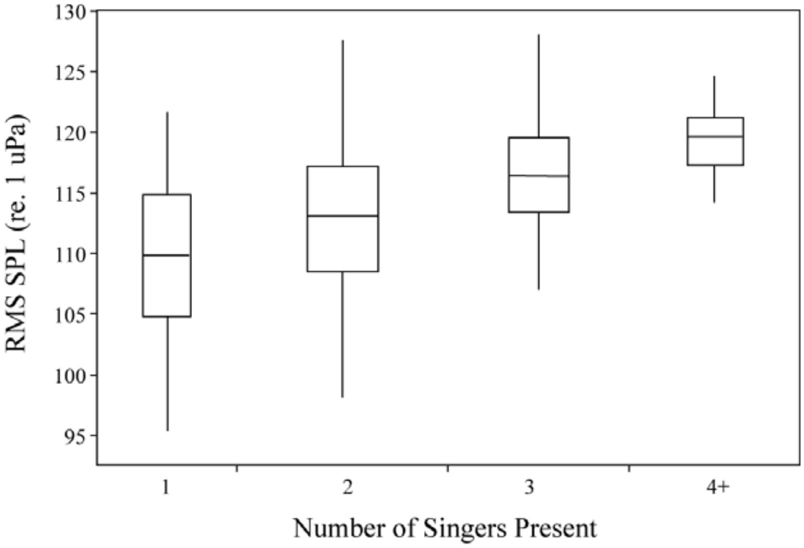

Figure 2.9. Quantile boxplots of average RMS sound pressure levels (re. 1 μPa) per number of singers detected at Location 1 (linear regression: $R^2 = 0.27$, $p < 0.0001$). RMS levels increase by an average of 3.3 dB per additional singer.

Table 2.2. Mean root-mean-square (RMS) sound pressure levels (SPL) levels ± SD per number of singers. RMS levels were measured for the first four minutes every half hour for a subset of data in which the number of singers present was quantified for only the pop-up in which the RMS measurements were made (pop-up at Location 1, n=136).

NUMBER OF SINGERS	SAMPLE SIZE	MEAN RMS SPL	SD
1	27	109.04	7.67
2	41	113.16	6.62
3	35	115.78	5.36
4	33	119.23	2.89

DISCUSSION

Data from this study reinforce the conclusion that more male humpback whales sing at night than during the day. When compiling data from 35 days of recording, significantly fewer males were singing during the Daylight period than during the Dark period. The change in the number of singers appears to be tightly correlated with the onset of twilight. Furthermore, a diel pattern in singing activity is evident at different times in the breeding season, despite a decrease in the average number of singers per day. Therefore, it appears that the tendency for males to sing more often at night occurs irrespective of the overall density of singers. However, there was an effect of singer density on the transition time in singing activity, with more singers becoming active during twilight periods when singer density was higher.

In the previous study by Au et al. (2000), in an effort to characterize singing activity levels over the course of a breeding season and on a daily cycle, the authors measured sound pressure levels received at one hydrophone placed approximately 0.8 km offshore in 13 m of water. While they recorded higher SPLs at night, they could not distinguish whether this was due to individual singers moving closer to the hydrophone area, whether individual singers were singing more loudly at night, or whether there was an increase in the number of singers.

In our study, measured RMS sound pressure levels were also higher during Dark periods compared with Daylight periods. The combination of these data with the singer count data confirm that an increase in sound pressure levels recorded at night is due to the increase singing activity of humpback whales in the area. Additionally, we interpret our results as a clear indication of increased numbers of males singing at night, rather than changes in distribution or movements of singers. In the current study, we believe that were there singers further offshore during the day, they would have been detected. Conservative estimates of detection range for a singing humpback whale in our study area yield distances of over 6 km between source and receiver. Using the generic sonar equation, we can calculate the distance at which a male's song would be below the ambient noise level. Given that our ambient noise level is approximately 94 dB, if we assume a transmission loss of 20 * log r (for spherical spreading), then a male producing song units of 170 dB would be detected above the background noise out to a range of 6.31 km. In practice, this has been confirmed during boat-based surveys. Singing individuals observed anywhere near the array were clearly audible on all four pop-ups, suggesting that a singer could be at least 6 km from the furthest pop-up (at one extreme end of the array) and still be reliably detected. Therefore, a conservative estimate of the detection area of our array can be constructed using a 6 km radius from the outer pop-ups (Figure 2.10).

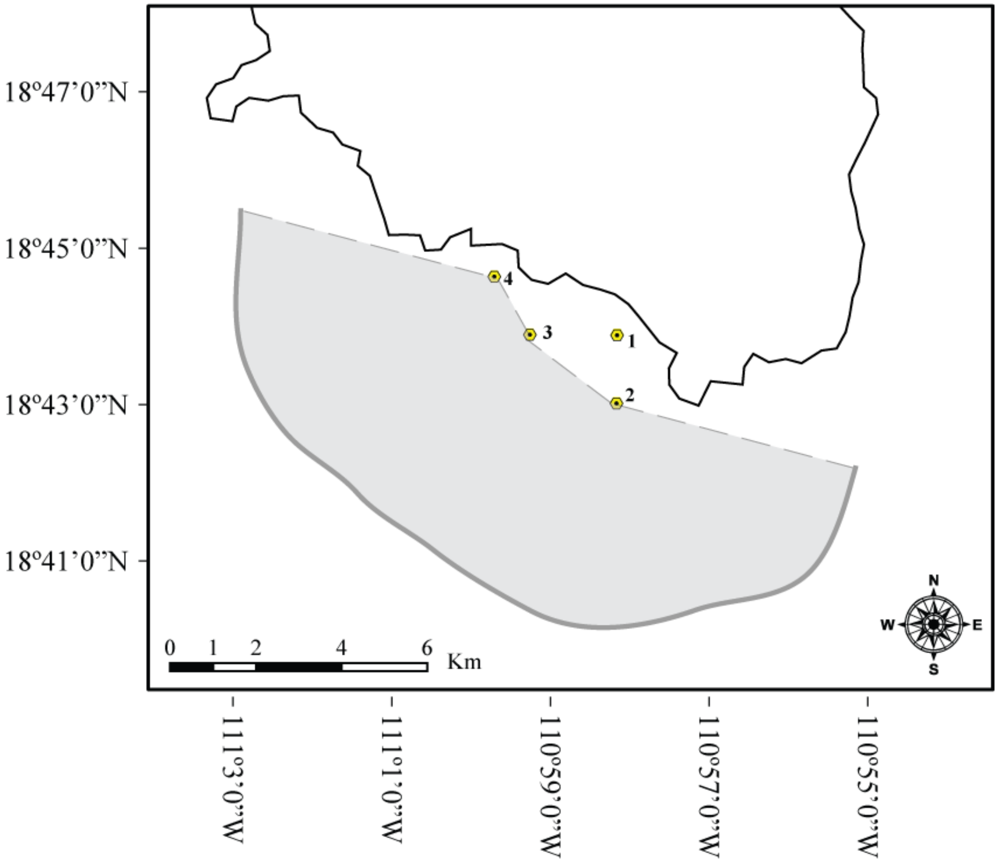

Figure 2.10. Isla Socorro, showing the array area and conservative estimate of detection range, based on a 6 km radius from each of the outer pop-ups. Pop-ups are indicated by hexagonal dots, the minimum detection area is indicated by the gray shading.

This detection range covers waters that are well over 500 m (ca. 300 fathoms) deep. Although the distribution of males singing in "offshore" waters in the breeding areas is not well studied, several studies have indicated that males preferentially sing in near-shore, shallow waters. Aerial survey data collected off the Hawaiian Islands (Mobley et al. 1999) found that 64% of all humpback whale sightings occurred in waters less than 100 fathoms (182 m) deep. In a prior study incorporating both theodolite as well as acoustic data to focus on singer distribution, Frankel et al. (1995)

found that 50% of the singers were located in waters greater than 100 fathoms. However, the density of singers was higher within 100 fathoms (0.62 singers/ km^2 within 100 fathoms as opposed to 0.17 singers/ km^2 outside of 100 fathoms), and only 20% of singers were found outside the 200 fathom (360 m) isobath. The detection range of our array covers this range of ocean depths off Isla Socorro. Therefore, if there were higher numbers of "offshore" singers during the day, we would have detected them. Thus, we conclude that our results reflect the fact that more males began singing at night, and not that more singers moved into the array's detection area.

For other balaenopterid species, diel variation in vocal activity seems to be linked with prey distribution. In some locales, blue whales exhibit a marked increase in calling at night, which seems to be inversely correlated with their deep-diving foraging behavior during the day (Stafford et al. 2005, Wiggins et al. 2005). Blue whales may increase calling rates with diminished feeding effort, or they may be advertising in an area where feeding has been productive, or perhaps calling and feeding are directly linked if individuals are engaged in surface lunge feeding at night (Stafford et al. 2005). Similarly, fin whales also exhibit a marked increase in vocal activity at night (Clark unpublished data**)**. Croll et al. (2002) suggest that male fin whales sing in association with prey aggregations, perhaps as a long-distance advertisement to attract females.

For humpback whales, however, singing in low latitudes is not linked with foraging activity, as prey resources in the breeding areas are essentially absent (Clapham 1996). Therefore, the selective pressures affecting the diel pattern in humpback chorusing may be distinct from those affecting other balaenopterids. In terrestrial animals, such as songbirds, the marked increase in song output just before dawn may be due to the advantages conferred by environmental conditions. Over many terrestrial temperature regimes and habitat types, sound propagation is on average highest in the early hours of the morning due to the interactions between temperature, humidity and wind (Henwood & Fabrick 1979). This may provide an advantage to individuals who make use of that time window to participate in the dawn chorus, therefore there would be significant selective pressure for this behavioral trait. In the ocean, however, it is unlikely that similar factors could be influencing song activity levels. Oceanic conditions such as temperature, salinity, and current movements do not change as rapidly as terrestrial conditions, and therefore would not provide an advantage to cetaceans singing at night.

If humpback song functions to attract mates, as has been hypothesized (Payne & McVay 1971, Clapham 1996), it would benefit males to spend more time singing either when females are more actively evaluating song or when females are more likely to become receptive (and it is conceivable that one is liked to the other). In some species of spontaneous ovulators, circulating levels of reproductive hormones vary on a diel cycle, which suggests that the onset of ovulation may be governed by circadian rhythms. In mammals, a surge in luteinizing hormone (LH) stimulates the release of a mature ovum, initiating the period of fertility in females (Hadley 1996). In the house mouse, both the preovulatory surge in LH and ovulation occur during the dark period (Bronson 1979), presumably coincident with the period in which females may be most mobile and have the highest chances of encountering a mate. In humans, controlled studies have demonstrated that the LH surge is significantly more likely to occur during nocturnal hours than during daylight hours (Cahill et al. 1998). While mice and humans have reproductive systems that are quite different from those of cetaceans, it may be possible that the onset of ovulation in female cetaceans also varies on a diel basis. While several authors have examined hormone profiles of cetaceans in captivity (Sawyer-Steffan et al 1983; Robeck et al. 2005a,b), with the aim of improving captive reproduction, diel patterns in hormone secretion do not seem to have been examined. We predict that cetaceans may also show a nocturnal surge in preovulatory hormones; if patterns of ovulation are conserved across cetacean species, then studies on captive animals may elucidate this hypothesis.

Although few studies have documented interactions between females and singing males, no studies have attempted to do so during the nighttime periods when singing activity is at its peak. Theory based on avian breeding systems suggests that, for species in which males can pair during the night, nocturnal song output is increased greatly relative to those species in which individuals do not pair or attract mates at night (Hutchinson et al. 1993). These models are thought to explain patterns of nocturnal singing activity in species such as the common nightingale (Thomas 2002). It may well be that in humpback whales, females are spending more time assessing singers at night than during the day, and this has generated higher selective pressure for males to engage in singing activity after dark. This may also explain why, despite many attempts to determine whether singing males attract females, few studies have documented it. All studies to date that have attempted to track interactions between singing males and other individuals have been conducted during daylight hours (Darling et al. 2006, Darling & Berube 2001, Tyack 1981). To thoroughly evaluate whether male song functions to attract females, it may be necessary to track singers and the individuals that join them during the night. Alternatively, effort could be focused on tracking female movements to evaluate whether they spend more time in the vicinity of singers after dark. In either case, if male humpback whales are using song to attract females, we predict that more of these interactions are occurring during nighttime hours. .

Finally, given that males divide their reproductive effort between multiple breeding tactics, it may simply be the case that males focus their reproductive effort during daylight hours on those tactics for which visual cues may be more important (Au et al. 2000). One well-known humpback breeding tactic is the use of aggressive competition within groups of males for a position near a focal female. Males within these groups often exhibit visual threat displays (Tyack and Whitehead 1983). For example, males may assume exaggerated S-shaped postures, inflate their ventral pleats, and blow bubbles (pers. obs.). If these signals provide the opportunity for males to assess competitors and therefore mediate intra-sexual interactions, then participation in competitive groups may be restricted to daylight hours. In this case, males may be shifting from the visual modality to the acoustic modality as daylight fades. Increased singing at night may therefore be a byproduct of this "modality-shift". This hypothesis predicts that males will spend less time in competitive groups at night, which has not been tested to date.

Currently available data do not allow us to distinguish between these hypotheses, and it is possible that they are not mutually exclusive. However, the fact that males sing more during nighttime hours indicates that there is a reproductive benefit to displaying acoustically during this period. It may be that males singing at night attract female consorts, and the pairs are then interrupted during daylight hours as other males move in to challenge and compete with the prior singer. Or, it may be that females evaluate potential mates based on a variety of criteria, which include not only song production but other behaviors as well. Assessment of paternity in our breeding population (Cerchio 2003, Cerchio et al. 2005) has demonstrated that reproductively successful males participate in a multiple mating tactics, only one of which is singing. It is clear that if we are to better understand the function of song in humpback whales, more effort will need to be focused at the time when males are singing the most: at night.

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CHAPTER THREE:

MALE-MALE INTERACTIONS IN HUMPBACK WHALES: DOES SONG PLAY A ROLE?

ABSTRACT

The function of song has been well-studied in numerous taxa, particularly among songbirds, and has been shown to play a role in mediating both inter-sexual and intra-sexual interactions in a variety of ways. Humpback whales are among the smaller number of mammals who sing, but the function of song in this species is very poorly understood. While one predominant hypothesis is that song may function in mediating male-male interactions, the mechanism by which this may occur has never been explored. In this study, we examine the song sequences and movement patterns of 13 focal singers to determine whether their behavior differs in the absence vs. presence of other singing males. Song pattern analyses revealed that males significantly increase the rate at which they switch themes in the presence of other singers ($p = 0.029$), and there is a trend towards increasing relative song versatility as well ($p=0.077$). Monte Carlo techniques were employed to determine whether additional singers thematically overlap the songs of focal singers more than expected by chance. While this was not the case for the overall sample ($p=0.401$), when pairs were examined individually, it was found that singers overlapped significantly more than expected in three cases. Additionally, location and movement pattern analyses reveal that singing males approach one another ($p=0.0148$) and end up closer ($p = 0.0123$) than expected by chance. The current sample was not large enough to

determine whether song characteristics (e.g., thematic overlap) are employed by singers to mediate spatial response (e.g., close approach), but the data suggest that male humpback whales may, at times, alter their song presentation to direct interactions with particular individuals.

INTRODUCTION

Males employ vocal displays towards reproductive means in a wide variety of species. Commonly among birds, anurans, and insects, males use song (or calls) to either attract females, compete with rival males, or both (Searcy & Andersson 1986). In an inter-sexual context, males may attract females with higher singing rates (Payne 1983) or calling rates (Sullivan 1983), larger repertoires (Searcy & Yasukawa 1990), or increased song versatility (Ammer & Capp 1999). In an intra-sexual context, males may use song to mediate territorial disputes, and song characteristics may predict the escalation of aggression via matching (Krebs et al. 1981), coordinated bout switching (Kramer & Lemon 1983), or switching rate (Simpson 1985; Molles & Vehrencamp 1999; Vehrencamp 2001). In addition, males may use song to distinguish between familiar individuals (e.g., neighbors) vs. unfamiliar individuals (Molles & Vehrencamp 2001). Song is less common among mammals, but in a few species song may also function in territory defense (Mitani 1988) and mate attraction (Bradbury 1977; Davidson & Wilkinson 2004).

In some species, males may also use different songs in different contexts (Trillo & Vehrencamp 2005). For example, mate attraction songs may be longer or more complex than those used in resolving intra-sexual conflicts (Catchpole & Slater 1995), or males may use different vocalizations when interacting with males vs.

females (Davidson & Wilkinson 2004). Additionally, the continuity, or timing, of song production may vary depending on its function. Males who are defending territories or engaging in intra-sexual competition may have longer pauses between songs to facilitate listening to a rival's response, while songs may be more continuous if directed at females, who generally do not respond acoustically (Ince & Slater 1985).

While song used as a display to females may be broadcast as an omni-directional signal, one important feature of song used in intra-sexual competition is the ability to direct it at specific rivals. This may be particularly important, for example, in territorial species, where males may be motivated to engage in acoustic competition with particular intruders, or distinguish between interactions with well-established neighbors and unfamiliar individuals. Considered within a broad framework, if song is used to mediate direct male-male competition, then one should be able to observe signs of acoustic interaction between singing individuals. Demonstrating interaction requires measuring a response of one individual relative to the actions of another. In general, males may vary song output along two different axes to acoustically "point" at specific receivers: via timing, or pattern (Todt & Naguib 2000). Timing responses may take the form of alternating or overlapping vocalizations with the other individuals, while pattern responses may take the form of song matching, or a combination of the two may occur (Todt & Naguib 2000).

The song system of humpback whales has been a topic of particular interest to behavioral ecologists for many years, yet we still understand very little about its function. The production of a long, elaborate song display is one of several alternative tactics used by males during the breeding season (Clapham 1996). Humpback song is particularly unusual because males progressively change their songs throughout the breeding season, and all males within an ocean basin incorporate these changes into their own songs (Payne et al. 1983; Cerchio et al. 2001). This form of synchronous rapid cultural evolution is relatively rare: the village indigobird (*Vidua chalybeata,* Payne 1985) and yellow-rumped cacique (*Cacicus cela vitellinus,* Trainer 1989) are among the few similar examples. A number of hypotheses have been proposed as to the function of song, including: a migratory beacon (Clapham & Mattila 1990), a mechanism by which to stimulate of ovulation in females (Baker & Herman 1984), and a method of sonar used to locate conspecifics (Frazer & Mercado 2000). However, the complexity in song structure suggests the influence of strong sexual selection, and the hypotheses that have gained the most prominence are that: i) song is used to maintain spacing between males (Frankel et al. 1995) ii) song is used establish and maintain dominance hierarchies (Darling 1983), or iii) that song is used to attract females (Winn & Winn 1978; Tyack 1981). Female behavior is very poorly understood, due to the difficulties in finding and identifying them on the breeding grounds, and there is as yet little evidence for female choice relative to singers (though see Medrano 1994; Darling & Berube 2001). Due to this, recent literature has emphasized the potential role of song in mediating interactions (be they competitive or otherwise) between males.

If humpback whales indeed use song to mediate intra-sexual interactions, then we would expect to find evidence of measurable responses of singing individuals relative to one another. On the other hand, if song functions primarily as male advertisement or mate attraction, then we would predict little change in the singing behavior of one male with respect to the presence of another.

Measuring acoustic interaction between humpback whales is not a trivial task. First, males sing continuously (Payne & McVay 1971), with phrase duration being extremely stable both within and between individuals (Cerchio et al. 2001). Second, all males within a population sing from the same ‘repertoire’, so that all phrase types are essentially shared among all individuals (with some rare exceptions). Third, males sing with ‘eventual variety’, that is, they repeat the same phrase type multiple times before switching to a new phrase type. Identifying acoustic response in eventual variety singers can be more difficult than in species that sing with immediate variety (Todt & Naguib 2000). Finally, humpback song is generally hierarchically structured, such that individuals sing different phrase types (or themes) in a similar order in successive cycles.

There is, however, variation both within and between the songs of individuals, and the function of this variation has not been explored. While the overall timing of song components is very stable, males may vary the number of times phrase types are repeated (and therefore the length of a theme), and to some extent may vary the order in which themes are sung.

The goal of this study is to evaluate the hypothesis that male humpback whales use song to mediate intra-sexual interactions. If males are interacting with one another acoustically, then we expect to see measurable changes in their song presentation in the presence of potential rivals as compared to when they are singing alone. To this end, I have analyzed the song sequences of individual males to test for changes in the rate of switching between themes and the relative song sequence versatility in the two acoustic contexts. Because switching rate and song versatility may be conventional signals that function differently among species (Molles 2006), and we have no *a priori* information as to how these variables may be used, if at all, in humpback whale song, we will test for either an increase or decrease in these measures. However, while a change in either of these variables may indicate an acoustic response on the part of one singer to another, this alone is not sufficient to indicate interaction. If males are directing their song towards one another, we also predict that the level of thematic overlap between the song sequences of two singers should be higher than expected by chance. Finally, we will also test whether singing males approach one another or vary their movement patterns with respect to one another. If singers are interacting, we predict that males will not be moving randomly, but will direct their movements towards one another.

METHODS

Humpback whales were recorded off Isla Socorro, México, during the breeding seasons of 2005 and 2006. Acoustic data were collected using autonomous recording units ("pop-ups"), developed by the Bioacoustics Research Program, Cornell University (Clark & Clapham 2004; *www.birds.cornell.edu/brp*). Six units were deployed from 2 March – 12 April in 2005, and five units were deployed from 1 March – 11 April in 2006. In both seasons, units were deployed 1.5-2 km apart in a W- or zig-zag pattern, along a 6 km stretch of coastline (Figure 3.1). Seafloor depth ranged from 35-100m at sites of deployment.

In 2005, three units recorded with a sampling rate of 2 kHz, and three recorded at a sampling rate of 16 kHz. The latter three were retrieved on 21 March and redeployed in the same locations on 22 March 2005. All units recorded continuously, 24h/day. In 2006, all five units recorded at a sampling rate of 16 kHz, from 0600h-2000h daily. After retrieval of the units each season, data were synchronized to ± 1 ms and merged to yield of total of 1000h of 6-channel recordings (2005) and 575h of 5-channel recordings (2006).

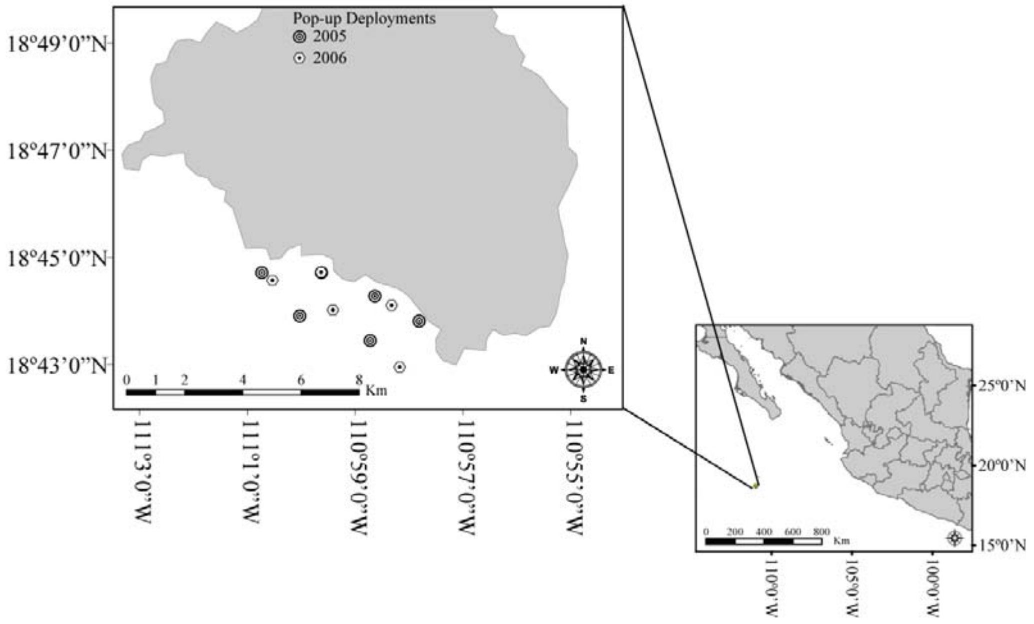


Figure 3.1. Isla Socorro, Revillagigedo Archipelago, México. Locations of the autonomous recording units (pop-ups) for the two analysis periods are indicated by circles (2005) and hexagonal dots (2006).

Data Review and Selection for Analysis

Data were reviewed visually and aurally using the software package Raven (Charif et al. 2007). Spectrograms were generated for both datasets (512 pt FFT or 1024 pt FFT, Hamming window, 50% overlap), and singing activity was quantified by counting the number of singers present in the 10-minute period at the beginning of every hour.

Based on the initial review of the data, hourly samples in which one singer was detected followed by an hourly sample in which more than one singer was detected were flagged for further examination. Periods for evaluation of potential interactions between singers were chosen using the following criteria: only one singer was audible within the array for a minimum of 45 minutes, after which time a second male began singing in the area. Over 40 periods were flagged and reviewed completely to determine whether the first audible singer (hereafter referred to as the "focal") was non-intermittent (so that there would be no possibility of switching "focals") and whether both singers were sufficiently close to the array to enable consistent acoustic detection. Thirteen periods were ultimately chosen for subsequent analysis, including two periods in which a second singer started singing less than 45 minutes after the onset of the focal. One of these periods includes a quartet rather than a dyad.

Song Pattern Analyses

Overall song structure for 2005 and 2005 was determined based on examination of boat-based recordings collected in the field. Spectrograms were generated for multiple individuals across each season and song structure each year was characterized at the level of phrase types (e.g., themes).

A total time window of 90 minutes was chosen for analysis, which includes up to 45 minutes in which the focal singer was the sole singer audible across the array (hereafter referred to as the "BEFORE" period), and up to 45 minutes in which the focal and one or more other singers were present (hereafter referred to as the "DURING" period). Each 45-minute block was divided into three 15-minute time windows to enable more discrete temporal resolution.

For each of the thirteen analysis periods, phrase structure was continuously delineated and annotated for both members of the dyad (or quartet in one case) using the software program Raven. Three parameters of song pattern were evaluated: 1) switching rate, 2) song versatility, and 3) overlap (or matching).

Switching rate: defined as the number of switches from one phrase type to another divided by the total number of phrases – 1. Switching rate was calculated over each 15-minute window for the focal singer in both the BEFORE and DURING periods, as well as for the second singer in the DURING period.

Relative song versatility (J´): a modified version of the Shannon Index (Zar 1999):

$$J' = -(\Sigma\, p_i \log p_i / \log k)$$

where p_i is the proportion of observations found in category *i*, and *k* is the number of categories (i.e. phrase types). The numerator is commonly referred to as H´, which is the Shannon Index for diversity. The denominator is a measure of the maximum diversity possible for data with *k* categories. Therefore *J´* is a calculation of the observed diversity as a proportion of the maximum possible diversity. *J´* can be also considered as a measure of evenness or homogeneity of the overall sample. The resultant outcome is a quantity between 0 and 1, with 0 indicating very low homogeneity (i.e., some categories are particularly over- or under-represented in the sample), and 1 indicating complete homogeneity or evenness (i.e., all categories are equally represented in the sample).

Relative song versatility was calculated for each singer independently. Because humpback song changes over time, and different numbers of themes may be included in the song at different points throughout the season (or in different seasons), it was important to calculate the maximum possible diversity with respect only to the themes that were present in an individual's song at the time of recording. Therefore, the maximum possible diversity varied between singers; the calculation of *J´*, which represents the observed diversity as a proportion of the maximum diversity, accounts for this difference among singers. *J´* was calculated for each singer, for 15-minute segment and averaged over the BEFORE and DURING periods.

Thematic overlap (e.g. matching): Because humpback song is continuous (i.e. pauses between phrases are not longer than pauses between notes), cyclical (Payne & McVay 1971), and all males generally sing the same themes, it was not possible to test for song matching by traditional methods. Therefore, the following Monte-Carlo procedure was implemented for each dyad:

1. The observed level of overlap between the song sequence of the focal singer and that of the second singer was calculated by evaluating the theme each singer was singing across a series of time slices, the interval of which was equivalent to one-half the minimum phrase length recorded for a member that dyad.
2. The song sequence of the second singer was shifted in time a random amount so that the new start time for the second singer fell within the original *BEFORE* period of the focal singer. The proportion of overlap was calculated for the newly aligned sequences. This process was performed 1000 times to generate a "null" distribution of overlap – essentially, the distribution expected if the song sequences of the two males were completely independent of one another.
3. The mean expected proportion of thematic overlap was calculated from the null distribution of each dyad.
4. The probability of the observed occurrence of overlap within each null distribution was calculated both for the initial 15-minute period after the onset of the second singer, and for the overall 45-minute DURING period.

Statistical Analyses:

Standard statistical analyses were performed using the software packing JMP (SAS Institute, 2007). Switching rates and relative song versatility proportions were arcsine transformed. Because it was not clear whether one would predict an increase or a decrease in switching rate and versatility in the presence of another singer, two-tailed paired t-tests were conducted to test: 1) whether the focal singers changed their switching rates or relative versatility between BEFORE and DURING period, 2) whether there was an 'immediate' change in either parameter as measured in the 15-minute window immediately before and immediately after the onset of the second singer, and 3) whether the focal and second singers differed significantly in either parameter.

Proportion of thematic overlap were calculated for each dyad for initial 15-minute window after the onset of the second singer, as well as for the overall 45-minute DURING period. A one-way Wilcoxon-Rank Sum test was used to test for an increase in observed levels of overlap as compared against the null expectation, derived from the hypothetical mean for each pair as calculated via the Monte Carlo simulations. The probability of the observed level of thematic overlap occurring by chance was also determined for each dyad independently from their null distribution.

Movement Pattern Analysis

Two-dimensional positions of singers were calculated using the difference in time-of-arrival of song units across multiple channels. To ensure precision of calculated positions, up to 10 different song units within 60s were located. The median position within this cluster was used for subsequent analysis. In most cases, dispersion around the median was reduced to less than 100m in the x- and y-axes, leading to highly precise positioning.

Each singer's position was calculated up to every 2.5 minutes, when possible. Surfacing events and movements out of the array area occasionally decreased the ability to calculate positions at this fine-scale time resolution, however, if the singer was within the array area, nearly continuous tracking was possible over the length of the analysis period.

Interpolated tracks were generated for all singers using ISRAT (I. Urazghildiiev), and were both plotted and animated to observe movement tracks of singing whales in detail.

Three movement parameters were calculated based upon the positions of the two singers in the dyad: 1) their initial separation distance, 2) their ending separation distance, either when one male stopped singing or at the end of the 45-minute analysis period, 3) the change in distance between the two singers. In addition, each singer's overall pattern of movement was quantified via calculation of their meander ratio.

Separation distances: In order to test whether singers were distributed non-randomly with respect to one another, their three movement parameters were compared against a null distribution of separation distances generated using locations of non-interacting singers. To generate this null distribution, acoustic data in 2005 and 2006 were reviewed for periods in which only one singer was audible within the array for at least 45 minutes, thereby enabling the calculation of a singer's position when he was not possibly interacting with any other singers. Eighteen different singers were selected for this analysis, and in all cases they were recorded on different days or after at least one hour of silence, to minimize the possibility that the same individual was used more than once. The location of each singer was calculated at the beginning and end of a 45-minute period. The null distribution of separation distances between non-interacting singers was generated by calculating the starting and ending distances between all possible combinations of these 18 singers, for a total of 153 pairwise combinations.

The three observed movement parameters were then compared against the mean parameters calculated from the nulls distribution to test whether simultaneously singing individuals were more closely distributed than expected by chance, or whether they approached one another more than expected by chance.

Meander ratio: defined as the total distance traveled divided by the straight-line distance between an individual's start and end position. An individual who is traveling in a straight line will have a meander ratio of 1, while an individual who is milling will have a meander ratio much higher than 1. Although singers were located up to every 2.5 minutes, their distance traveled was calculated over 5 minute bins to minimize potential errors associated with oversampling tracks. The meander ratio was calculated over the entire 45-minute BEFORE and DURING period for the focal singer, and over the entire DURING period for the second singer.

Statistical Analyses:

Standard statistical analyses were performed using JMP. All movement analyses were tested using non-parametric tests. The observed starting distances, ending distances, and change in distance between singers were tested against the means derived from the null distributions using Wilcoxon-Rank Sum tests. A Mann-Whitney test was employed to determine whether change in distance between singers was related to the cessation of song by one of the individuals.

RESULTS

Thirteen periods were selected for analyses, although not all periods were suitable for both song pattern and movement analyses. See Table 3.1 for a summary of periods and data analyzed. Twelve of 13 periods were composed of dyads, while one period (11 March 2005 am) was composed of a quartet. Two different pairs within the quartet were included in the analysis (focal + Singer 2, Singer 3 + Singer 4). The other pairwise interactions in this group will be discussed but were not included in the statistical analyses.

In three cases, either the focal male or the second singer stopped singing within the 45-minute analysis period. These are included in the analysis. In a fourth case, the focal singer stopped singing within 4 minutes after the onset of the second singer. This dyad was not included in the analysis because the short period in which both were vocally active precluded any comparison of the focal's behavior BEFORE vs DURING, or any calculation of thematic overlap between the two singers. Both males continued singing in all other cases, though in several instances one or both singers subsequently stopped singing after the end of the analysis period (Table 3.1).

Table 3.1. Sample used for each analysis. Thirteen periods were selected for analyses, but not all periods could be included in all analyses. Singers were excluded from certain analyses if: 1) the onset of the second singer happened too quickly to enable robust calculation of BEFORE period parameters for the focal individual, 2) song sequences could not be continuously delineated to allow for robust calculation of song parameters. Sample sizes for individual analyses therefore ranged from 10-13. An additional dyad (23 March 2006) is not included in these analyses due to an extremely short temporal overlap between the focal and second singer (the focal male quit singing within 4 minutes after the onset of the second male).

	Switching Rate	Relative Versatility	Thematic Overlap	Separation Distances	Meander Ratio	OUTCOME
2 Mar 05	X	X	No	X	X	Singer 2 quits
7 Mar 05	X	X	X	X	X	Focal quits
10 Mar 05	X	X	No	X	No	Continued
11 Mar 05 am (Focal + Singer2)	No	No	X	X	X	Continued
11 Mar 05 am (Singer 3 + Singer 4)	No	No	X	X	X	Continued
11 Mar 05 pm	X	X	X	X	X	Continued
13 Mar 05	No	No	No	X	No	Continued
2 Mar 06	X	X	X	X	X	Continued
3 Mar 06	X	X	X	X	X	Continued
14 Mar 06	No	No	X	X	X	Continued
19 Mar 06 am	X	X	X	X	X	Continued
19 Mar 06 pm	X	X	X	X	X	Continued
23 March 06	No	No	No	No	No	Focal quits
30 Mar 06	X	X	X	X	X	Continued
31 Mar 06	X	X	X	X	X	Singer 2 quits

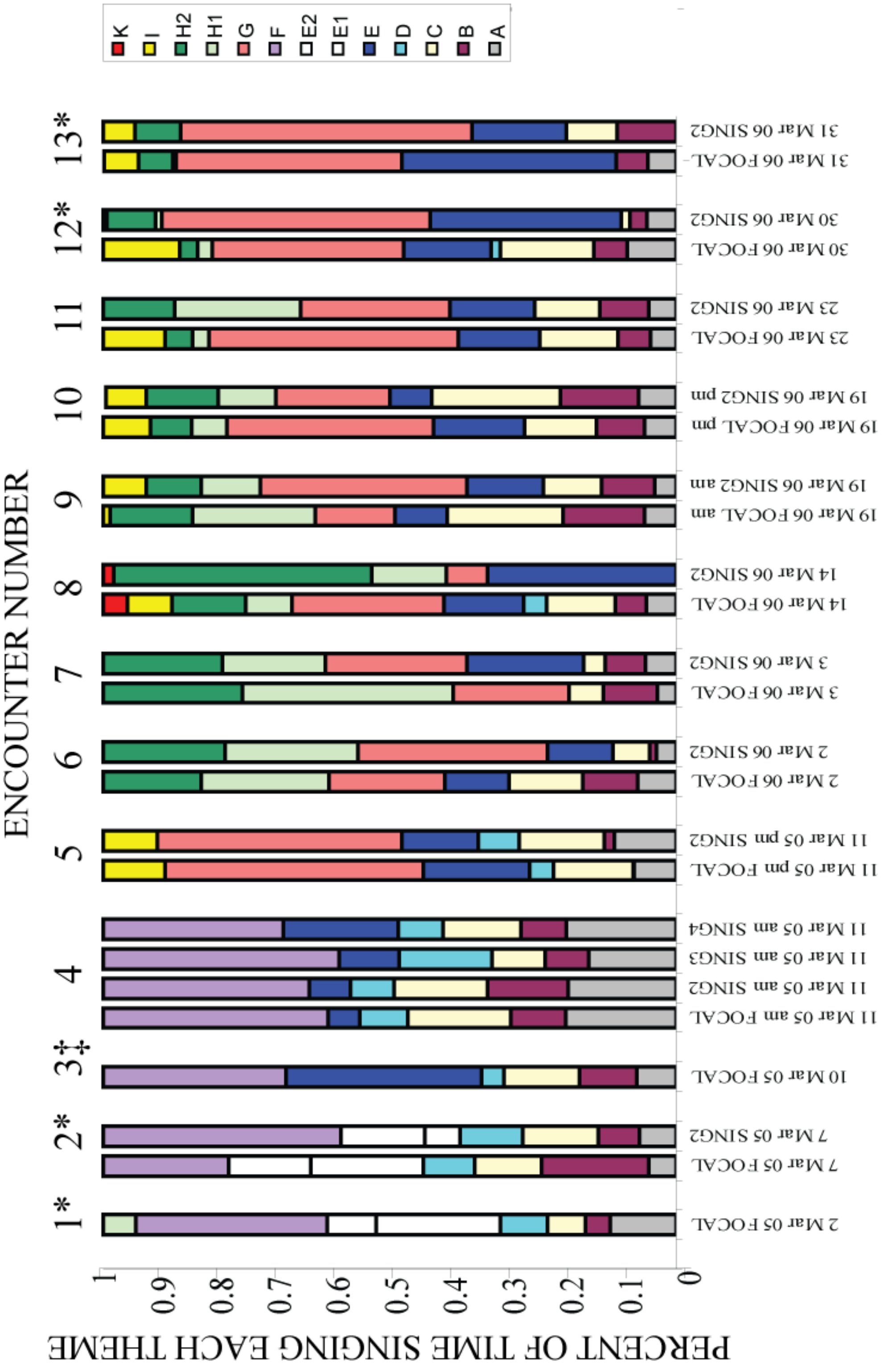
Song Pattern Analyses:

A total of 13 different phrase types (themes) were identified in 2005 and 2006, although no more than 10 were present in the population-wide song at any one time. Two themes (E1 and E2) converged into one (E) by mid-March 2005, and one theme (F) was split into two (H1 and H2) between 2005 and 2006. Overall song composition for each singer who sang for more than 15 minutes is displayed as the proportion of time spent singing each theme (Figure 3.2). Variation between singers with respect to proportional representation of each theme, as well as the evolution and loss of themes over time, can be observed. One pair of males (14 March 2006) appeared to sing a theme that was unique within this sample (theme K), although a more thorough analysis of the population-wide song composition at the time of their recording would be necessary to evaluate the significance of this observation.

Switching Rate and Versatility:

Mean switching rate for focal singers in the *BEFORE* period was 0.11 ± 0.035, while their mean switching rate in the *DURING* period was 0.14 ± 0.033. The increase in switching rate was significant (2-tailed test: t-Ratio: 2.65, df = 8, p=0.029). However, the focals' mean switching rate in the 15-minute period immediately prior to the onset of a second singer was not significantly different than mean switching rate within the 15-minute period immediately following the onset of the second male (2-tailed test: t-Ratio: 0.175, df = 8, p=0.866) (Table 3.2). Mean switching rates of the focal singer in the *DURING* period were not significantly different from those of the second singer (t-Ratio: -0.099, df = 7, p= 0.924).

Figure 3.2. The overall thematic composition of each male's song sequence, represented as the proportion of time spent singing each theme. Focal and second singers are paired by date for all cases in which the song sequences of both males could be analyzed. The song sequences of the second singers could not be analyzed on 2 March 2005 or 7 March 2005. The 11 March 2005 am sample represents a quartet of singing males. Thirteen themes were identified in total over the two-year study period, but no more than 10 were ever present in the song at one time. Differences between individuals and changes in song composition over time can be observed.


ENCOUNTER NUMBER
PERCENT OF TIME SINGING EACH THEME
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
1*
2*
3‡
4
5
6
7
8
9
10
11
12*
13*
2 Mar 05 FOCAL
7 Mar 05 FOCAL
7 Mar 05 SING2
10 Mar 05 FOCAL
11 Mar 05 am FOCAL
11 Mar 05 am SING2
11 Mar 05 am SING3
11 Mar 05 am SING4
11 Mar 05 pm FOCAL
11 Mar 05 pm SING2
2 Mar 06 FOCAL
2 Mar 06 SING2
3 Mar 06 FOCAL
3 Mar 06 SING2
14 Mar 06 FOCAL
14 Mar 06 SING2
19 Mar 06 FOCAL am
19 Mar 06 SING2 am
19 Mar 06 FOCAL pm
19 Mar 06 SING2 pm
23 Mar 06 FOCAL
23 Mar 06 SING2
30 Mar 06 FOCAL
30 Mar 06 SING2
31 Mar 06 FOCAL
31 Mar 06 SING2
K
I
H2
H1
G
F
E2
E1
E
D
C
B
A

Mean relative song versatility ranged from 0.61 to 0.91 between males. Mean relative song versatility for the focal singers in the *BEFORE* period was 0.74 ± 0.066, while mean versatility for the same singers in the *DURING* period was 0.78 ± 0.087. There was a trend towards an increase in relative versatility after the onset of a second singer, though it was not significant under a two-tailed t-test (t-Ratio: 2.03, df = 8, p = 0.077). Mean song versatility for the focal singers in the 15-minute period immediately before (0.75 ± 0.091) and after (0.76 ± 0.112) the onset of the second singer was not significant (t-Ratio: 0.601, df = 8, p=0.565), nor was there a significant difference between relative versatility in the song sequences of the focal male and the second singer (t-Ratio: -0.33, df = 7, p=0.749, Table 3.2).

Thematic Overlap

The observed proportion of thematic overlap between the song sequences of two simultaneously singing males ranged from 0.05 to 0.33. The mean proportion of overlap expected under a null distribution ranged from 0.13 to 0.32 for different dyads. An overall test of observed vs. mean expected overlap revealed no significant difference for either the overall 45-minuted period (p=0.401) or the period immediately following the onset of the second singer (p=0.653).

However, analysis of the probability of occurrence of the observed level of overlap within each dyad’s null distribution revealed that some pairs of singers were overlapping significantly more than expected given the focal singers prior song sequence (Table 3.3). Thematic overlap was significant in three pairs of males and there was a trend towards significance in a fourth pair in one of the two analysis windows.

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Table 3.2. Mean switching rates and relative song versatility measures for focal and additional singers. Superscripts in the rows indicate corresponding columns. Focal singers show a significant increase in switching rate in the presence of an additional singer, and a trend towards an increase in relative song versatility, but only when measured over the entire analysis period. Switching rates and relative song versatility were not significantly different in the period immediately before and after the onset of an additional singer, nor were they different between the focal singers and the second singers.

		Mean ±SD^a	Mean ±SD^b	*p* value
SWITCHING RATE	Focal singer: BEFORE^a (45 minutes) vs DURING^b (45 minutes)	0.12±0.035	0.14±0.033	*0.029**
	FOCAL singer: BEFORE^a (15 minutes) vs DURING^b (15 minutes)	0.13±0.046	0.13±0.026	0.866
	Focal singer^a vs. Singer 2^b: DURING (45 minutes)	0.14±0.033	0.13±0.021	0.924
RELATIVE VERSATILITY	Focal singer: BEFORE^a (45 minutes) vs DURING^b (45 minutes)	0.74 ±0.066	0.78±0.087	*0.077*
	FOCAL singer: BEFORE^a (15 minutes) vs DURING^b (15 minutes)	0.75±0.091	0.76±0.112	0.565
	Focal singer^a vs. Singer 2^b: DURING (45 minutes)	0.78±0.087	0.76±0.083	0.749

Table 3.3. The probability of observing the measured level of thematic overlap between two singers, based on the null distributions of overlap generated by 1000 Monte Carlo simulations for each dyad. Thematic overlap was measured, and a null distribution of expected overlap if the two song sequences were independent was generated for both the entire 45-minute analysis period, as well as for the initial 15-minutes after the second male initiated singing. Two dyads overlapped in theme sequence significantly more than expected based on their null distributions when evaluating the entire 45-minute sequence; one dyad overlapped significantly in the first 15 minutes, but this effect diminished in the longer time window. One pair showed a trend towards thematic overlap, but this was not significant at the 0.05 alpha level.

SINGERS	*p value:* 45 MINUTE COMPARISON	*p value*: 15 MINUTE COMPARISON
7 Mar 2005		0.352
11 Mar 2005 am FOC_2	0.063	*0.006**
11 Mar 2005 am 3_4	*0.001**	0.81
11 Mar 2005 pm	0.279	0.621
2 March 2006	*0.006**	0.502
3 March 2006	0.553	0.586
14 March 2006	0.479	0.188
19 March 2006 am	0.96	0.938
19 March 2006 pm	*0.077*	0.321
30 March 2006	0.881	0.959
31 March 2006	0.425	0.32

Movement Pattern Analyses:

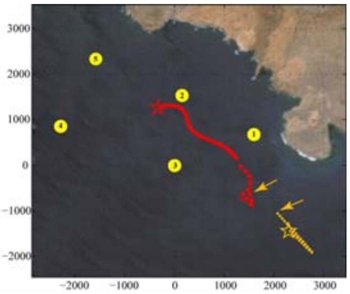
Individual singers displayed a wide variety of movement patterns (Figure 3.3). Some were essentially stationary, while others were clearly traveling during the analysis period. Several singers appeared to approach one another, while others maintained inter-individual distances.

Separation Distances:

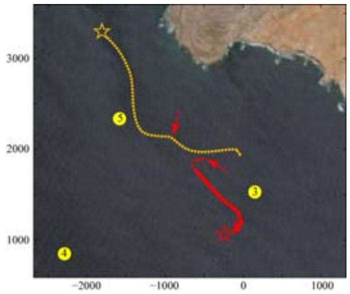
The mean starting separation distance based on the null distribution generated from pairwise comparisons of non-interacting singers was 3059 ± 1517.9 m. The mean ending distance based on the null distribution was 3420 ± 1590.7 m and the mean change in distance was 362 ± 702.5m (Figure 3.4).

Observed starting separation distance between two simultaneous singers ranged from 830 – 7504 m. Second singers did not begin singing closer to focal animals than expected based on the null distribution (Wilcoxon-Signed Rank: p= 0.1138). Observed ending distances between simultaneous singers ranged from 538 - 8456 m. This was significantly closer than expected based on the null distribution (Wilcoxon-Signed Rank: p=0.0123), and the change in distance between singers was significantly lower than expected (Wilcoxon-Signed Rank: p=0.0148), indicating that singers approached one another more than expected. The change in distance between singers was significantly correlated with whether one male ceased singing (Mann-Whitney: Z=-2.18, p=0.0293, Figure 3.5).

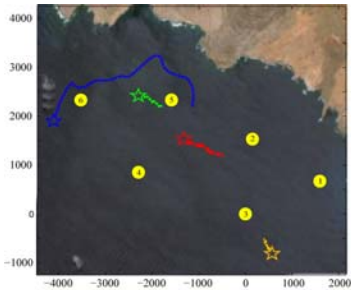
Figure 3.3. Interpolated tracks of singer movements generated from positions calculated every 2.5 minutes or more. Pop-ups are indicated by yellow circles. Focal singers are in red, the solid line indicates the singer's track in the BEFORE period, the dotted line indicates his track in the DURING period. Second singers are in orange. Stars represent the initial position for each singer. In three cases, one singer quit singing in the DURING period; the position of each singer at the time when one quit is indicated by the arrows, the color of the arrow indicates which singer quit singing. On 11 March 2005 am, a quartet was tracked. In this case, singer 3 (blue) and singer 4 (green) are both indicated.



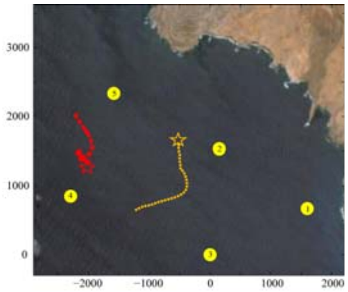
2 March 2005



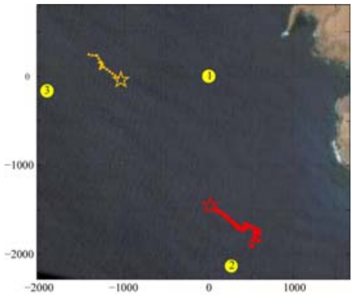
7 March 2005



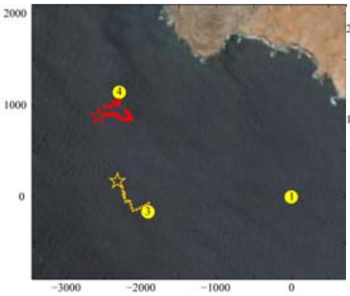
11 March 2005 am



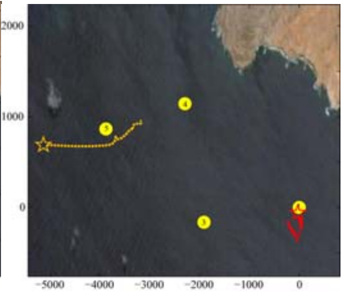
11 March 2005 pm



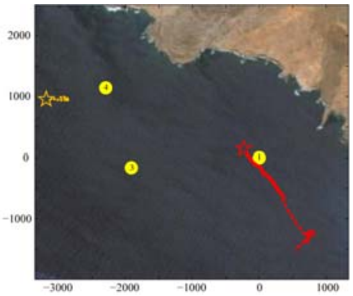
2 March 2006



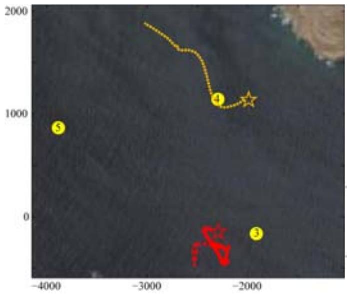
3 March 2006



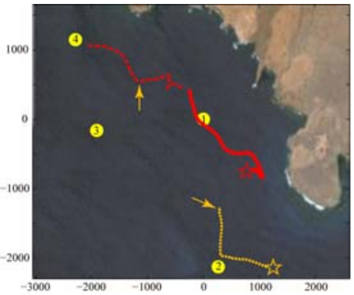
19 March 2006 am



19 March 2006 pm



30 March 2006



31 March 2006

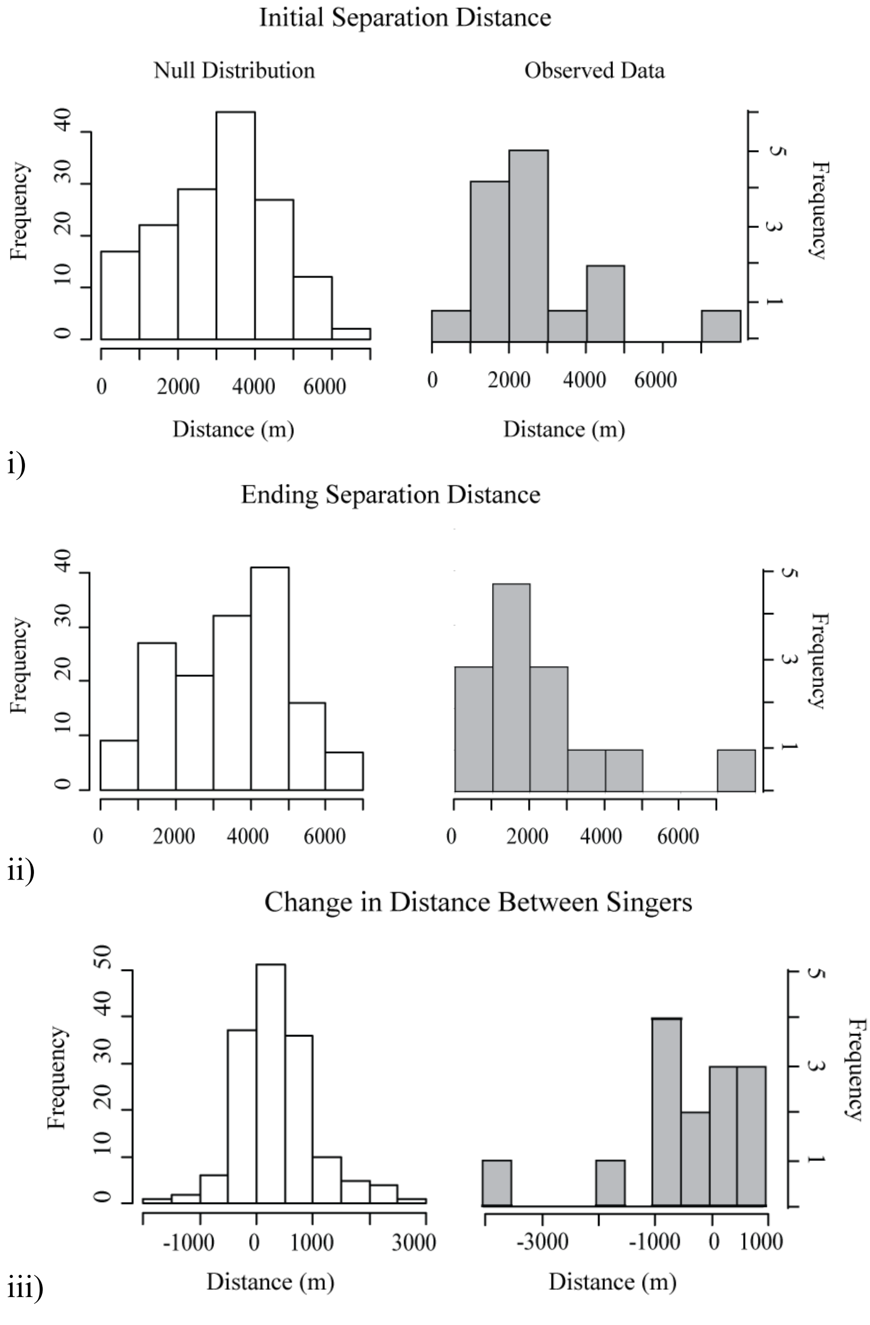


Figure 3.4. Distributions of i) starting separation distances, ii) ending separation distances, and iii) change in distance between two singers. The distributions on the left are null distribution created from pairwise comparisons of locations calculated for 18 solo singers, the distributions on the right are observed distances between pairs of simultaneous singers. .

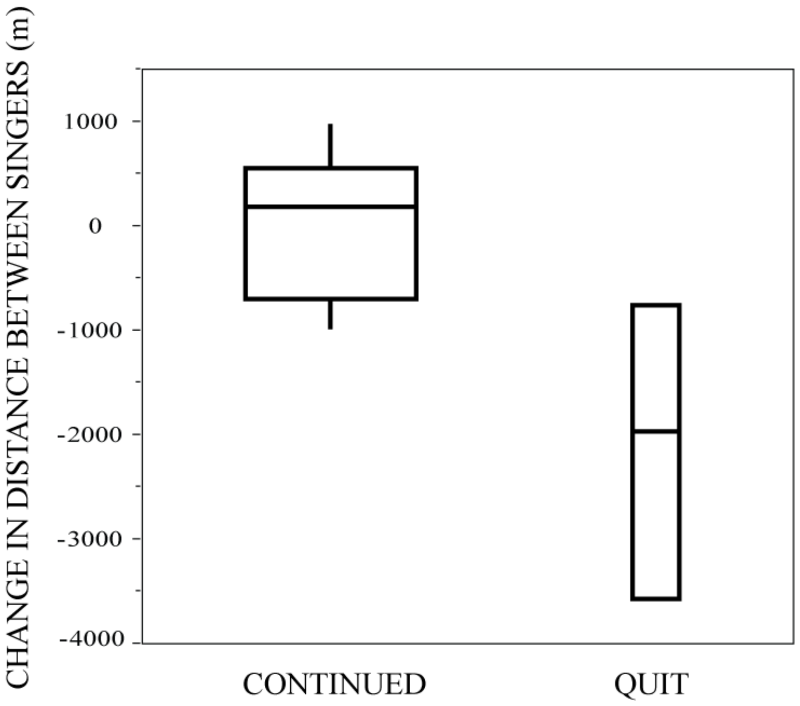


Figure 3.5. Box plot of the change in distance between the focal and second singers during the 45-minute analysis period, grouped by whether both males continued singing throughout the analysis, or whether one male quit singing. The mean change in distance between members of dyads in which both males continued to sing was 16.5 ± 685.78 m, while the mean change in distance between dyad members when one singer quit singing was -2101.7 ± 1410.69 m. The change in distance was significantly different for the two categories (Mann Whitney: z= -2.18, p=0.0293)

Meander Ratio:

The average meander ratio for focal singers was 2.35± 1.65 for the *BEFORE* period and 2.69 ± 1.46 for the *DURING* period. This difference was not significant (Wilcoxon-Signed Rank: p=0.275). However, second singers were significantly more directional (average meander ratio: 1.49 ± 0.41) than focal singers (Mann-Whitney:=, Z=2.69, p= 0.007).

DISCUSSION

Significance of song pattern analyses

Previous studies of humpback whale song have proposed that males may use song to mediate interactions between themselves and other singers, but a mechanism by which this might be accomplished has never been investigated. This study provides a first attempt to evaluate and quantify ways in which males may use song to direct interactions with one another. The song pattern analyses suggest that males do respond to the presence of another singer in the area by increasing the rate at which they switch between themes as compared to when they are the sole audible singer. Correspondingly, there was a trend towards an increase in relative versatility in the presence of another singer, which indicates that singers become less repetitive and instead cycle through all of the themes in their song sequence more quickly than when they are alone.

Although males may increase their switching rate in the presence of other singers, it is difficult to say whether this constitutes actual 'interaction' between singers. The analysis of thematic overlap, however, suggests that, in at least a few cases, males do indeed vary their song output to direct their song at one another. The observed level of thematic overlap was significantly higher than expected for 3 of 11 dyads, and a trend towards higher overlap was observed in a fourth dyad. In one case, the period of higher overlap appeared to be predominant in the first 15 minutes after the second male began singing, while in the other cases, matching occurred either over the course of a longer time-span, or during a different period within the 45-minute analysis window.

Significance of movement analyses:

A predominant hypothesis within the humpback literature suggests that singing males are distributed throughout their display range and that song may function to enable singers to maintain spacing between one another (Frankel 1995). Analysis in which multiple singers were simultaneously tracked seemed support this idea: singing males were never found to approach one another at distances of less than 4 km, and only one pair was documented to maintain a distance of 2 km between individuals. In the majority of cases, singers were found to move away from one another. Supporting evidence was presented in that singers were found to surface oriented away from other singers, rather than towards them (Frankel 1995). This study, along with others in which song was played back to singers, who subsequently avoided the playback, suggested that singing males are not directly interacting with one another on the breeding grounds, but are instead avoiding each other.

The current study provides evidence contrary to this commonly held belief. While the mean distance between singers when a second male initiated singing was not closer than expected based on the null model, the overall results suggest that males do approach each other significantly more than expected, and several singers approached each other to distances of less than 1 km. These "close approaches" (within the range of ~ 600 m) seem to be correlated with the cessation of singing by one of the two individuals, while males who did not approach to the same distance continued to sing.

These results suggest that, at least in some cases, singing males may be directly competing with one another. We cannot determine what happened after one male ceased to sing, and whether close approaches between singers ever result in direct aggression is unknown. At a minimum, however, the outcome of these approaches can be interpreted as the displacement of one singer by another. The fact that not all singers interact in this way suggests that various factors may be involved in the decision to approach and ultimately displace another singer. These factors may include familiarity with the other singer, assessment of competitive ability, or motivation to continue singing within a particular region of the breeding ground.

Additionally, the focal and second singers differ significantly in their overall pattern of movement. The movement pattern of focal singers was in general less directional, in some cases more similar to milling or stationary behavior, while the second male to begin singing in the area moved in a more directional manner. This biological significance of this difference in movement pattern is not clear at this time, but we can conclude that the movements of singing males do appear to be influenced by the presence of other singers.

How are acoustic interactions and movement patterns related?

Given that singing males are occasionally approaching one another, resulting in the cessation of singing on the part of one of the singers, the next logical question is whether these approaches are mediated by the preceding acoustic display on the part of one or both singers. If so, this could provide a mechanism by which intra-sexual selection could shape song characteristics in humpback whales. Song parameters such as switching rate, versatility, and matching have been shown to be important in intra-sexual interactions in a variety of other species, notably songbirds. Any or all three of these measures have been correlated with differing degrees of agonism between males, and may predict the likelihood of escalation or attack. In some cases, an increase in these measures is correlated with a context of increasing aggression (i.e., song-type matching is correlated with strength of response in the great tit (Krebs et al. 1981), switching rate and versatility increase as distance from playback decreases in Carolina wrens (Simpson 1985), while in other cases, the reverse is true (i.e. banded wrens decrease switching rate and diversity when increasing aggression (Molles 2006)).

To further understand the significance of the acoustic interactions between singing humpback whales, and to really evaluate the function of song in the context of intra-sexual selection, it is necessary to test whether instances of thematic overlap ("matching"), switching rate, or other song parameters are correlated with the probability of approach between two singers. The current sample is not large enough to allow robust analyses of this type, but the results of both the song pattern and the movement analyses suggest that: 1) some males are interacting directly with song, 2) some males are interacting directly via close approaches, and 3) more extensive analysis may enable us to determine whether acoustic interactions are important in mediating the spatial interactions.

Case-study: a quartet of singing males

Determining the length of time over which to analyze potential interactions between individuals is difficult, especially in a species where males may sing continuously for hours, and individuals may move in and out of acoustic range with one another in relation to other external factors (e.g. the presence of other individuals passing through the area). Additionally, most male-male interactions have traditionally been studied in dyadic form, because it is difficult to unravel more complex interactions (Todt & Naguib 2000). The analysis of the behavior of multiple individuals relative to one another in a chorus has rarely been undertaken. However, examination of these situations can sometimes be revealing, if quite challenging to analyze.

One of the analysis periods presented in the current study involved the ultimate presence of a quartet of singing males, rather than a dyad. This was discovered only during detailed examination, as initially the interaction was thought to represent only two singers. The pairs presented in the original analysis were the focal and second singer (consistent with other analyses), and also the third and fourth singer that started singing nearly simultaneously. The extent of the potential interaction between all members within this group is not evident in the previous analyses, both because all pairwise combinations of individuals were not included, and because the length of time over which the interactions appeared to occur was greater than the 45- minute analysis period.

The full sequence unfolded as follows (Figure 3.6):

4:40 am: Focal singer begins singing within the array

4:49 am: Singer 2 begins singing approximately 2800 m southeast

5:00 am: Singer 3 begins singing, approximately 2600 m west of the focal

5:01 am: Singer 4 begins singing, approximately 1400 m from the focal and Singer 3

5:43 am: Singer 4 moves towards Singer 3, approaches him to approximately 640 m, then continues to move directly towards the Focal.

6:10 am: Singer 2 stops singing.

6:12 am: Singer 4 stops singing.

6:25 am: The focal singer reverses course abruptly and heads towards Singer 3.

6:40 am: The focal and Singer 3 approach to a distance of 600 m, at which point Singer 3 stops singing.

6:41 am: The focal singer continues to sing for several more hours. He is the only singer remaining in the array.

The same Monte Carlo technique presented earlier was used to examine potential thematic overlap between each pair of singers, comparing each pair's song sequence independently. When all pairwise comparisons are examined, it appears that, in addition to the overlap noted between the focal and singer 2, and that between singer 3 and singer 4, there is also more thematic overlap than expected between the focal and singer 3, while all other pairs did not overlap more than expected (Table 3.4).

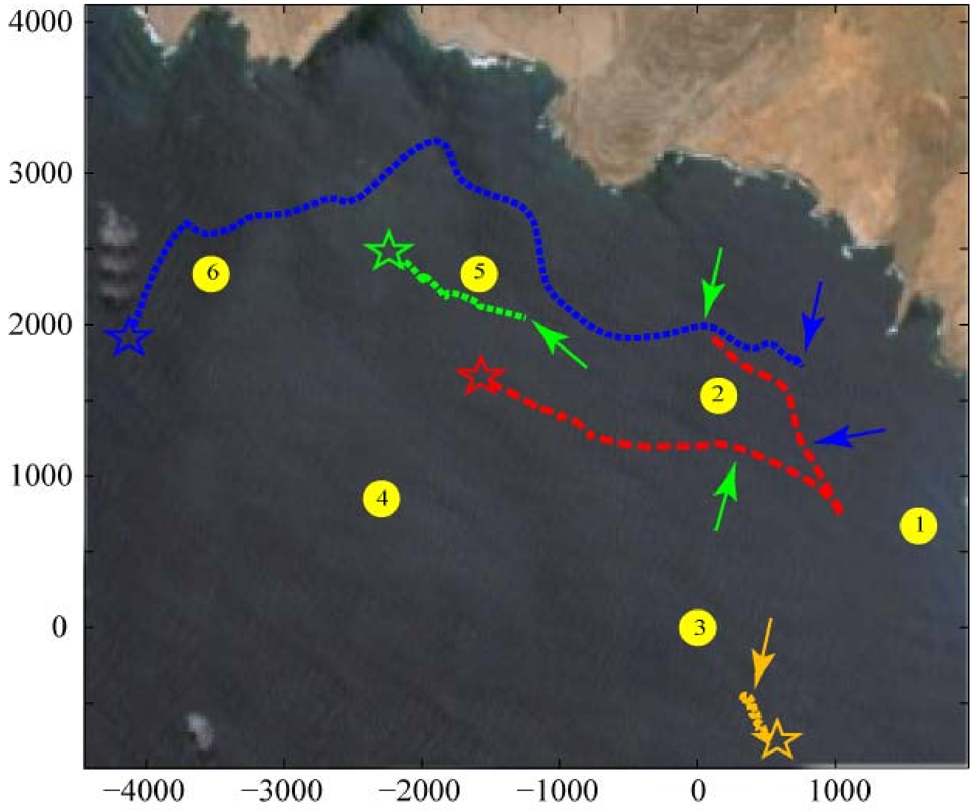


Figure 3.6. Four simultaneous singers (focal: red, singer 2: orange, singer 3: blue, singer 4: green) on 11 March 11 2005, tracked from the point at which each began singing, until the end of the analysis at 6:41am.

Yellow circles indicate pop-ups. Stars indicate starting positions for each singer. The position of each singer at the time when one quit is indicated by the arrows, the color of the arrow indicates which singer quit singing. For example, the green arrow pointing to the track of the focal singer (red), indicates where the focal was when singer 4 (green) stopped singing. Singer 2 and singer 4 stopped singing nearly simultaneously; therefore, additional orange arrows were not added to the tracks of the focal and singer 3. Over the course of the analysis period, all additional singers eventually stopped singing, with only the original focal singer remaining vocally active. The third singer (blue) moved across the array directly towards the focal male (red), who reversed course to approach singer 3. Singer 3 ceased singing when the two males approached to within 600 m.

Table 3.4. The probability of observing the measured level of thematic overlap between two singers, based on the null distributions of overlap generated by 1000 Monte Carlo simulations for each dyad within the quartet on March 11, 2005. Thematic overlap was measured, and a null distribution of expected overlap if the two song sequences were independent was generated for both the entire 45-minute analysis period, as well as for the initial 15-minutes of simultaneous singing. The results suggest that the second and third singers are thematically overlapping the focal singer more than expected by chance, and that the fourth singer is overlapping the third singer, while neither the third nor the fourth singers are matching the second singer's song, nor is the fourth singer matching the focal singer's song.

	p value: 45 MINUTE COMPARISON	*p value*: 15 MINUTE COMPARISON
FOCAL- SINGER 2	0.063	*0.006**
FOCAL – SINGER 3	0.326	*0.026**
FOCAL – SINGER 4	0.834	0.999
SINGER 3 – SINGER 4	*0.001**	0.081
SINGER 2 – SINGER 3	0.995	0.614
SINGER 2 – SINGER 4	0.691	0.613

The combination of these data suggests that singers within a multi-male group (e.g. chorus) may in fact be directing their attention at particular individuals, while ignoring others. The ultimate spatial interaction between singer 3 and the focal singer was not evident from the initial 45-minute analysis, but when examined over a longer time frame, it appears that singer 3 is directing his movements towards the focal male, who eventually responds by reversing his direction and directly approaching singer 3. In this case, this spatial interaction appears also to have been preceded by at least one period in which singer 3 was matching the focal singer's thematic sequence. Again, we do not know what ultimately becomes of singer 3, whether he remains in the area, or whether, once ceasing to sing, he moves out of the area entirely. It is possible that this interaction was a form of "acoustic challenge", which he ultimately lost. Further analyses of multi-male groups may prove to be enlightening with respect to the ability of singers to direct their attention towards particular rivals while avoiding others.

A note on "Singer-Jointer Interactions" previously discussed in the literature

It has been well documented in the humpback literature that singers are frequently "joined" by other individuals, whom are primarily males. The function of these joining events is unclear, and the duration of the interactions is overwhelmingly short. In a comprehensive study on interactions between singers and other individuals, "joining" events lasted on average 7.4 min, with a median length of 5 minutes (Darling et al. 2006). Extremely important to note is that most of these types of events involve a non-singing individual (or occasionally multiple individuals) approaching a singing male, who almost always stops singing during the interaction (Darling et al. 2006). Sometimes the "joiners" were males who were previously singing in the area, and sometimes they were not. Darling et al. (2006) proposed that these interactions may constitute some form of male coalition-building; although the pattern of those interactions might well fall into the category of intra-sexual display disruption that is sometimes observed on leks (Foster 1983), this was not considered as a hypothesis.

The analysis of singer behavior in the current study differs from that of Darling et al.'s analysis in two ways. First, in the aforementioned study, analysis of song patterns and potential acoustic interactions between singers was not conducted. Our data suggest that singers may be interacting with one another acoustically prior to or without approaching one another spatially. Second, only a small proportion of the interactions analyzed in Darling et al.'s study involved two simultaneous singers. The majority of the interactions observed were the result of non-singing individuals "joining" (or interrupting) singers, as well as some cases of singers ceasing to sing to join with other groups of whales. The previous authors were able to analyze potential interactions between simultaneous singers in nine cases. In 5 of these, it appears that one singer may have approached another, although it is not clear whether the two males continued singing in any of these instances.

While Darling et al.'s study highlights the importance of associations between males during the breeding season, the significance of these associations, and the role that song may play in mediating them, is not clear. The authors suggest that these brief associations between singers and a host of other individuals may reflect potential cooperative alliances, and that males may use song features to assess the degree of association between themselves and other singers (since humpback song is continually changing, two males that are present for the same amount of time may sing more similar songs than a male who has newly arrived to the breeding ground). However, it seems possible that these associations between non-singing and singing whales may instead reflect a form of competition in which males disrupt the display of their rivals. In any case, the interactions between simultaneous singers presented in the current study may be considered a separate category of interaction than the majority of those presented in Darling et al.'s study.

Summary and suggestions for future work:

It is clear that, despite several decades of research on humpback whale song, we have not advanced very far in unraveling the intricacies and the function of song in this system. While we have made great advancements in our understanding of the overall pattern of song sharing between populations and temporal change in song, we are still struggling to understand the role that song plays in mediating interactions between individuals (whether they be inter-sexual or intra-sexual interactions), and the selective pressures that may have led to such a complex, evolving song system. It may be that we have not yet analyzed humpback song within the appropriate framework. There are several reasons for this. It has been very difficult to study the behavior of individuals over long periods of time, but more importantly, is has been nearly impossible to study the behavior of multiple individuals relative to one another. Unlike many songbirds, where males may hold territories and be reliably located, or frogs, where many individuals within a chorus can be observed at once, the tools to simultaneously track and analyze the behavior of multiple individuals within a cetacean breeding system have not been readily available. As these tools become more widespread and their use more common, we can begin to evaluate interactions between individuals on a much broader scale than has previously been possible. In addition, it may be beneficial to draw more heavily from the wide body of literature that has been developed on other species. In particular, the analyses in this study present a first attempt to analyze humpback song using methods frequently employed in the analysis of songbird interactions. The hope is that these metrics may enable us to understand the dynamics of humpback song in a different context. The results of this study provide evidence that male humpback whales may be using their song to direct interactions with particular individuals. In addition, males may also direct or coordinate singing with other singers via other methods, such as by coordinated switching (Kramer and Lemon 1983). Also, among songbirds, different song types are sometimes used in different contexts (Trillo & Vehrencamp 2005), with certain song types being emphasized more in the context of escalated aggression. The song pattern analysis conducted in this study was a broad measure of "versatility", essentially measuring whether individuals sang all of the themes relatively equally within discrete time frames, or whether a smaller number of themes were emphasized at the expense of others. However, a more detailed analysis of the relative proportions of each theme within the context of solo-singing vs. dyad- or chorus- singing might be more revealing. Additionally, the combination of acoustic and spatial analyses will be necessary to evaluate the importance of intra-sexual selection on male song characteristics. In summary, although the sample of individuals examined in this study was relatively small, it appears that singing humpbacks may be interacting acoustically with one another, and hopefully the methods employed here will provide avenues for further exploration.

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CHAPTER FOUR:

SINGING TO HUMPBACK SINGERS: DO THEY RESPOND?

ABSTRACT

Playback experiments are relatively uncommon in cetacean studies, with the majority of playbacks focusing on the response of animals to anthropogenic noise. Few studies have investigated the responses of individuals to conspecific sounds; only three such studies have previously been undertaken with humpback whales and the response variables measured were restricted to spatial data and the determination of whether singing males ceased to sing. The current study evaluated both spatial as well as acoustic responses to the playback of humpback song to focal singing males. Of 12 trials included in these analyses, singers stopped singing in 3 cases. In two of these trials, the males approached the playback vessel to within 50m after ceasing to sing. In the other 9 trials, males continued to sing and significantly increased the overall evenness of their song sequences during the playback session ($p = 0.03$). There was a trend towards an increase in switching rate as well ($p=0.077$). The mean change in distance between singers and the playback vessel was 307 ± 379m ($n=11$ trials, excluding only the one in which the focal male quit singing immediately). This change in distance was not significantly different than expected based on a null model. Analyses of spatial movement patterns revealed no significant change in directionality during the playback trials ($p = 0.16$). Overall, the results of these playback

experiments revealed a greater response on the part of singers to the presentation of song that previously believed, and suggests that males do respond acoustically the presence of other singers.

INTRODUCTION

Humpback whales sing long, complex songs during the breeding season, but the function of this acoustic display remains unclear. Although many hypotheses have been proposed (Baker & Herman 1984; Clapham & Mattila 1990; Frazer & Mercado 2000; Darling et al. 2006), the predominant theories are that males sing to attract females (Winn & Winn 1978; Tyack 1981), or to establish and/or maintain dominance hierarchies between males (Darling 1983). It has also been suggested that males may use song to maintain inter-singer spacing (Frankel et al. 1995), which may be true, but is unlikely to be the driving selective pressure behind the evolution of the complexity observed in this song system.

Although the hypothesis that males use song to mediate intra-sexual interactions has been popular in the literature (Darling & Berube 2001; Darling et al. 2006), a mechanism by which this may be achieved acoustically has never been suggested. Among songbirds, it has been demonstrated for multiple species that males modify song presentation while interacting with potential competitors (Searcy & Andersson 1986; Todt & Naguib 2000). Some of the salient song variables include rate of switching between song types, temporal overlap of songs, and matching of song types (Molles & Vehrencamp 1999; Krebs et al. 1981).

While humpback song has never been analyzed in the same framework as avian song, the structure of humpback song suggests that the same types of acoustic variables could be used in mediating interactions between singers. Humpback song is traditionally recognized as following a hierarchical structure (Payne & McVay 1971), being composed of units that are grouped into patterns (phrases), which are repeated several to many times. A bout of similar phrases is referred to as a theme, and song sequences are composed of multiple themes, which are often repeated in the same order. An entire sequence of themes is considered to be a "song" by the classical definition (Payne & McVay 1971). In comparison to the terminology used in the avian literature, humpback whales can be considered to be eventual variety singers (Hartshorne 1956; Molles & Vehrencamp 1999). Although inter- and intra-individual variability in song features (such as number of phrase repeats within a theme, song sequence length, etc.) is widely recognized within the humpback literature (Frumhoff 1983; Helweg et al. 1998), little effort has been made to evaluate the context of this variability and its potential functional significance.

If males use song to mediate intra-sexual interactions, then it should be possible to detect changes in song features associated with different contexts. Yet whether or not males even respond acoustically to the presence of other singers has been previously untested. However, recent work suggests that males not only modify their song presentation in the presence of other singers, but may engage in thematic matching with nearby singers (see Chapter 3).

Playback experiments have been widely used to examine the responses of individuals to conspecific and heterospecific signals in a variety of taxa, especially anurans and songbirds (for a review, see Falls 1992). Within cetaceans, however, playbacks are still relatively uncommon; the logistical difficulties of conducting such experiments make controlled behavioral studies difficult at best (for a review of marine mammal playback experiments, see Deecke 2006). The majority of controlled-exposure experiments conducted to large whales have addressed the responses of animals to anthropogenic noise (Frankel & Clark 2000; Croll et al. 2001; Fristrup et al. 2003). Few studies have attempted to evaluate the response of individuals to conspecific sounds. Among these, two studies have demonstrated that right whales (*E. australis & E. glacialis,* respectively) respond to conspecific vocalizations (Clark & Clark 1980; Parks 2003), through approaches and/or increased vocalization rates. Only three playback experiments have been conducted to humpback whales to determine their responses to song and other types of vocalizations. One of these (Baker & Herman 1984), was an anecdotal study in which humpback song was presented to a group composed of a male escort and a mother with her calf; the group initially moved away and then later appeared unaffected by the playback. The other two studies found that humpbacks in various group compositions either avoided the playback of song (Tyack 1983), or appeared largely unaffected by song as compared to other vocalizations (Mobley et al. 1988). None of these prior studies investigated the acoustic response of singing humpback whales to song playback experiments, and generally concluded that humpback song does not act as an "attractant", based on spatial responses alone. However, failing to assess an acoustic response on the part of singing males greatly limits our ability to evaluate the function of song within this system. The current study utilizes playback experiments to further explore the idea that singing humpback males may be interacting acoustically with one another (as suggested by the results in Chapter 3). I test the hypothesis that singing males will respond acoustically to the presence of a nearby "acoustic intruder", as represented by the playback of song. Based on results of previous studies of natural encounters between singers, I predict that singing males will modify their rates of switching between themes as well as the overall evenness of their song presentation, in response to playbacks.

METHODS

Playback trials were conducted to singing humpback whales off Isla Socorro, México, during March and April 2006. Data were collected via boat-based observations and recordings, as well as via autonomous acoustic recording units ("pop-ups"). All boat-based work was conducted using a 5m inflatable Zodiac equipped with a 30 hp outboard engine. Song sequences were recorded from focal males using an HTI SSQ-94 hydrophone and a Marantz PMD 670 digital recorder sampling at 48 kHz. Identification photographs of singers were collected using a Nikon D100 digital camera fitted with a Nikon 70-200m lens. Shore-based observers aided in the location and tracking of singers, using Eagle Optics Ranger 10x40 binoculars, as well as a Lietz Sokkisha DT4 theodolite connected to a PC laptop, running the software program Pythagorus (Gailey & Ortega-Ortiz). Playbacks were conducted using Type J-11 transducer from the Naval Undersea Warfare Center, which has a frequency range of approximately 30 Hz – 10 kHz and is omnidirectional to 3 kHz.

Five pop-ups (Clark & Clapham 2004, *www.birds.cornell.edu/brp*) were deployed from 1 March – 11 April 2006, approximately 1.5 - 2 km apart in a zig-zag pattern, along a 6 km stretch of coastline (Figure 4.1). Seafloor depth ranged from 35-100m at sites of deployment. All units recorded at a sampling rate of 16 kHz, from 0600h-2000h (local time) daily.

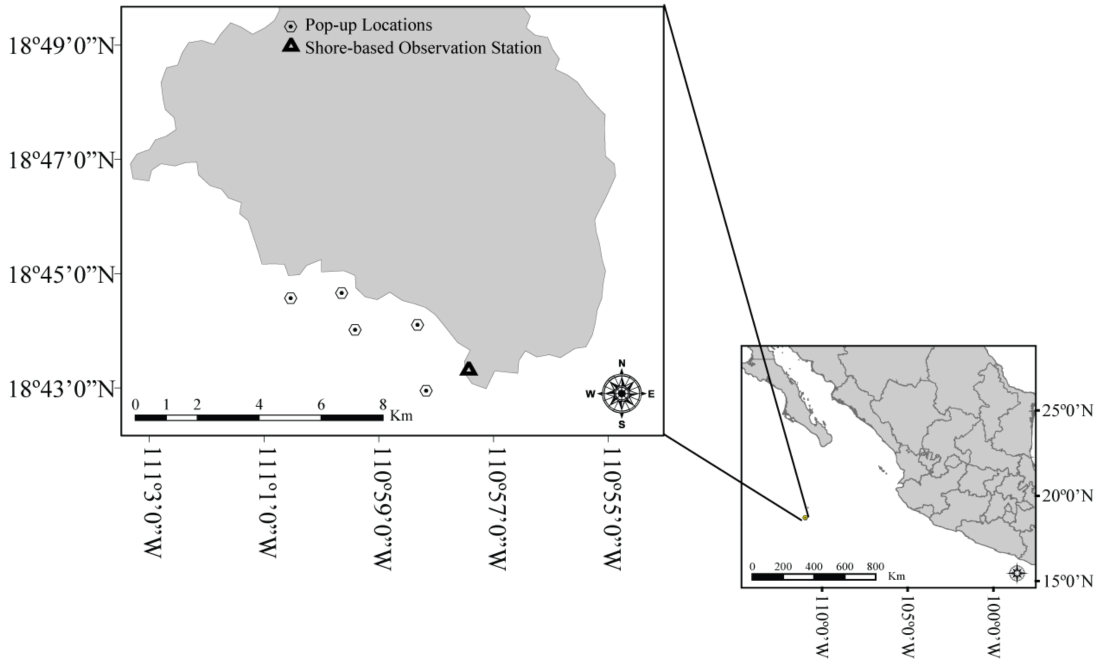


Figure 4.1. Isla Socorro, Revillagigedo Archipelago, México. Locations of the autonomous recording units (pop-ups) deployed from 1 March – 11 April 2006 are indicated by the hexagonal dots, and the location of the shore-based observation area is indicated by the dark triangle.

Acoustic Surveys and Location of Singers

Each morning during the 40-day pop-up deployment period (not including the day of deployment and the day of retrieval), weather conditions were assessed to determine whether boat-based work was feasible. The boat was deployed on 33 of 40 days. Prior to the departure of the boat, shore-based observers began scanning the array area from the shore station (Figure 4.1). Sightings and locations of all humpback pods were noted and group composition was determined when possible. Singing males are typically lone individuals, and are often distinguishable by their surface behavior (i.e., singers are often stationary or traveling slowly, surface at regular intervals, and blow 4-6 times before the terminal dive, upon which they usually lift their tail flukes). Potential singers were continuously tracked using the theodolite, with priority given to those individuals that were closer to the observation station.

Upon departure from the dock, the boat-based team moved into the array area and established contact with the shore-based observers. If no potential singers had been sighted by the shore team, the boat team acoustically surveyed the array area to determine whether singers were present. A hydrophone was deployed approximately every 2 km as the boat team moved from SE to NW across the array area. When singing was detected, the boat and shore teams worked together to locate the singing individual. If no singers were detected, the boat team collected data on other pods in the area, returning to the array throughout the day to survey acoustically for singers.

Focal Recording and Playback Stimulus Preparation

Once a singer was located within or near the array, the boat-based team began a systematic process to identify and acoustically record the individual. Humpback whales can be individually identified by the black-and-white patterns on the ventral surface of the tail flukes (Katona et al. 1979). When a singer surfaced, the boat team positioned itself behind him (without approaching) and photographed the tail flukes upon the terminal dive. These photographs were later compared to one another and to a catalog of all identified individuals from this breeding site, for assignment of identification numbers. After the singer dove, the boat slowly approached his location, and recorded his song sequence until at least his next surfacing cycle. Due to individual singer movements and/or weather conditions, it was sometimes necessary to reposition the boat and continue recording during more than one dive interval to obtain recordings with a high signal-to-noise ratio.

Once a sufficient acoustic sample had been collected (i.e., high-quality recording of the entire song sequence between surfacing bouts), the boat was allowed to drift while the playback stimulus was prepared. All playback stimuli were prepared using Raven (Charif et al. 2007). Spectrograms were generated (1024 pt FFT, Hamming window, 50% overlap) and the entire song sequence was examined. Three themes were chosen for the playback stimulus. An attempt was made to choose the same themes for every playback trial, but due to variation between individuals and variation due to temporal change in the song characteristics, this was not always possible.

In general, the selected themes included: 1) a theme with low-frequency notes and relatively little frequency variation, 2) a theme with mid-frequency notes and a pulsive frequency-modulated sweep, and 3) a theme with both high and low-frequency notes and relatively high frequency variation. A "new" song was constructed in which several phrases from each of these three themes were repeated for 3-minutes each, to produce a 3-theme, 9-minute song (Figure 4.2). An individual male's song contained between 6 and 10 themes, therefore, the stimulus created was one that simulated a low-diversity, highly repetitive song.

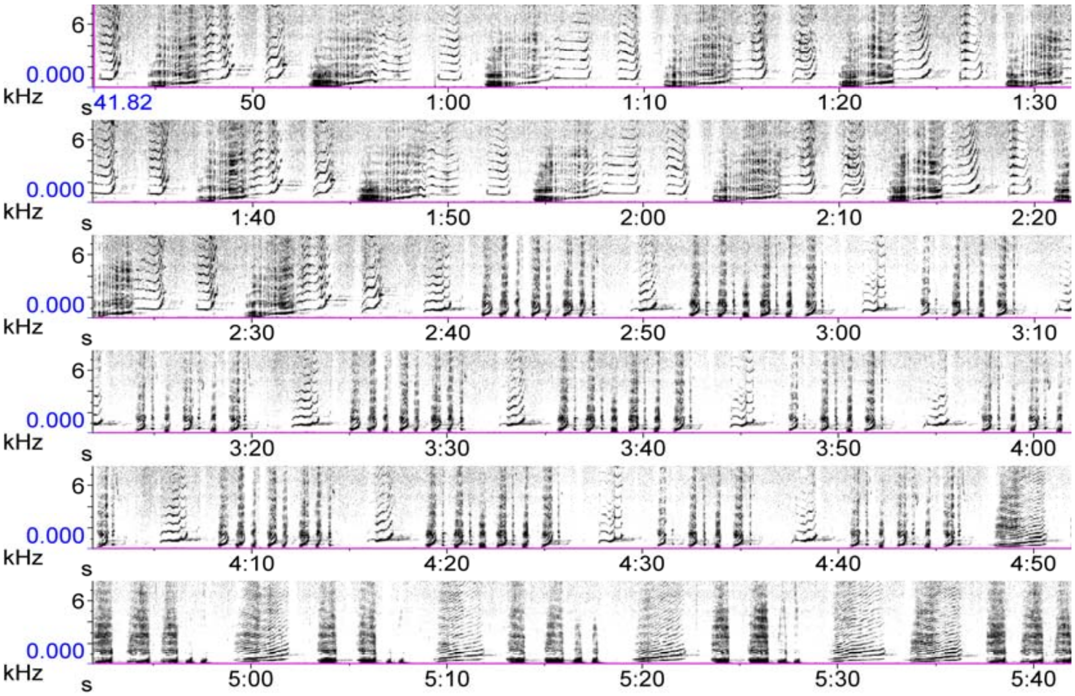


Figure 4.2. Sample spectrogram of playback stimulus created for Trial #10 (4 April 2006 am), showing the repetition of phrases of three different themes. The total playback stimulus was approximately 9 minutes, with the phrases of each theme being repeated for approximately 3 minutes.

Playback Experiments

Creation of the playback stimulus took approximately 25 minutes, after which time the shore-based observers aided the boat team in re-locating the focal singer, who was positively re-identified before an attempt was made to initiate playback. The boat team attempted to position itself within 500m of the singer and deployed the playback transducer over the side of the boat approximately 10-12 m depth. Playback was initiated within one minute of the terminal dive of the subsequent surfacing of the singer. This was done both to ensure that the singer's position relative to the boat was accurately known at the start of playback, and to ensure that all playback experiments were initiated in the same stage of the singer's dive cycle.

The playback stimulus was played a series of five times, for a total of 45 minutes (with the exception of the first two playback experiments, in which the stimulus was presented for only 20 minutes). During the playback period, the shore-based team assisted in tracking the focal singer and noting all surfacing activity. The entire playback trial was conducted regardless of whether or not the singer continued to sing.

Post-Field Data Review and Analyses

Upon returning from the field, pop-up data were synchronized to ± 1 ms and merged to yield of total of 575h of 5-channel recordings. Spectrograms were generated (1024 pt FFT, Hamming window, 50% overlap), and data were reviewed visually and aurally using the software packages Raven (Charif et al. 2007) and XBAT (www.xbat.org, Figueroa & Robbins 2008). Movement patterns and song sequences were analyzed for each of the focal singers for a 45-minute pre-playback period ("BEFORE"), and the 45-minute period during the playback experiment ("DURING").

Song Sequence Analyses

Three song parameters were calculated. These were switching rate, relative song "evenness", and the "playback theme" proportion.

Switching rate (SR): is the observed number of switches from one phrase type to another (N_s) divided by the total number of phrases (N_p) – 1.

$$SR = N_s / (N_p - 1)$$

Relative song evenness (J´): is a modified version of the Shannon Index (Zar 1999):

$$J' = \frac{-\sum_{i=1}^{k} p_i \log p_i}{\log k}$$

where k is the number of categories (i.e., phrase types, or themes), and p_i is the proportion of observations found in a category (i.e., the proportion of phrases of each theme for the BEFORE or DURING sequence). The numerator is commonly referred to as the Shannon Index for diversity, H'. H' is affected not only by the relative proportion of each theme within the song sequence, but also by the number of themes. Because the number of themes present in the song may change over the course of the breeding season (as humpback song temporally evolves in structure), a measure of evenness relative to the number of themes present for each individual enables comparison between individuals at different times within the season. Therefore, J' is a calculation of the observed evenness in the song sequence as a proportion of maximum possible evenness. The resultant outcome is a quantity between 0 and 1, with 0 indicating very low homogeneity (i.e., some themes are particularly over- or under-represented in the sample), and 1 indicating complete homogeneity or evenness among themes (i.e., all themes are equally represented in the sample).

Playback theme proportion: is the proportion of the BEFORE and DURING song sequences represented by the themes used in the playback stimulus. The total number of phrases in these three themes were counted for each period and divided by the total number of phrases for that period. The proportion of “playback themes” in the

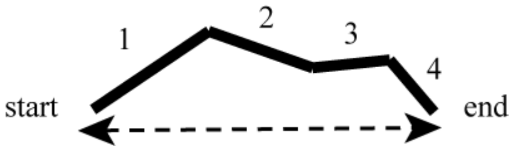
DURING period was compared with the proportion sang in the BEFORE period to determine whether singers increased their representation of playback themes in response to the stimulus.

Spatial Analyses

Focal singer locations (two-dimensional; x,y) were calculated in XBAT using time-of-arrival differences between channels song units (correlation sum estimation approach, Cortopassi & Fristrup, unpublished). To ensure precision of a singer's position, locations were computed for as many as 10 different song units occurring within a time span of ≤ 60s. The median position for this cluster of locations was used as the singer's position for subsequent analyses. In most cases, dispersion around the median was less than 100m in both the x- and y-axes, leading to highly precise singer positions. Each singer's position was calculated as often as 2.5 minutes, when possible.

Spatial parameters measured from the singer position data include meander ratio, the distance from the playback vessel at start of playback, the distance from the playback vessel at end of playback, and the change in distance between the singer and playback vessel.

Meander ratio (MR): the total distance traveled (sum of all tracks) divided by the straight-line distance between an individual's start and end position.



An individual who is traveling in a straight line will have a meander ratio of 1, while an individual who is milling will have a meander ratio much higher than 1. Although singers were located as often as every 2.5 minutes, their distance traveled was calculated over 5-minute periods to minimize potential errors associated with oversampling tracks. The meander ratio was calculated for the BEFORE and DURING periods.

Statistical Analyses

Standard statistical analyses were performed using the software packing JMP (SAS Institute, 2007). Switching rate, relative song evenness, and "playback theme" proportions were arcsine transformed. One-tailed, paired t-tests were conducted to determine whether focal singers increased their switching rates, relative song evenness, or proportion of playback themes between for the BEFORE and DURING periods. Two-tailed, paired t-tests were used to determine whether focal singers changed their meander ratio in response to playbacks. The change in distance between the focal singer and the playback vessel was compared to the mean (362 m) of a null distribution of change in distances between non-simultaneous singers (Chapter 3), to test whether singers changed their distance from the playback vessel more than expected by chance.

RESULTS

A total of 16 playback trials were conducted to at least 15 different singers from 6 March – 9 April 2006 (one singer was not identified). Twelve of those trials are included in this analysis. The first two playbacks were excluded because the playback stimuli were shorter than the rest of the trials (approximately 20 vs. 45 minutes). Two other playbacks were excluded because there were several other singers present and the focal animal's song could not be clearly distinguished.

In 3 of 12 trials, the focal singer stopped singing before completion of the 45-minute playback, and latency to song cessation ranged from 1 – 37 minutes (Table 4.1). In at least two of these trials, the focal individual approached the boat within minutes of ceasing to sing. In one of these cases when the focal singer approached, he surfaced parallel to the boat, approximately 20m away, and exhaled forcefully. In the second case, the focal male circled the loudspeaker underwater (observed from the boat), surfaced parallel to the boat ca. 50m away, and exhaled several times. In both of these close approach cases, the focal male subsequently moved away from the boat while the playback continued. In the third case of song cessation, it is not clear where the focal male went after ceasing to sing. However, 20 minutes after the trial, as the boat team was packing up the playback equipment, a different individual surfaced approximately 50m from the boat. It is not known whether this individual was present in the area during the playback.

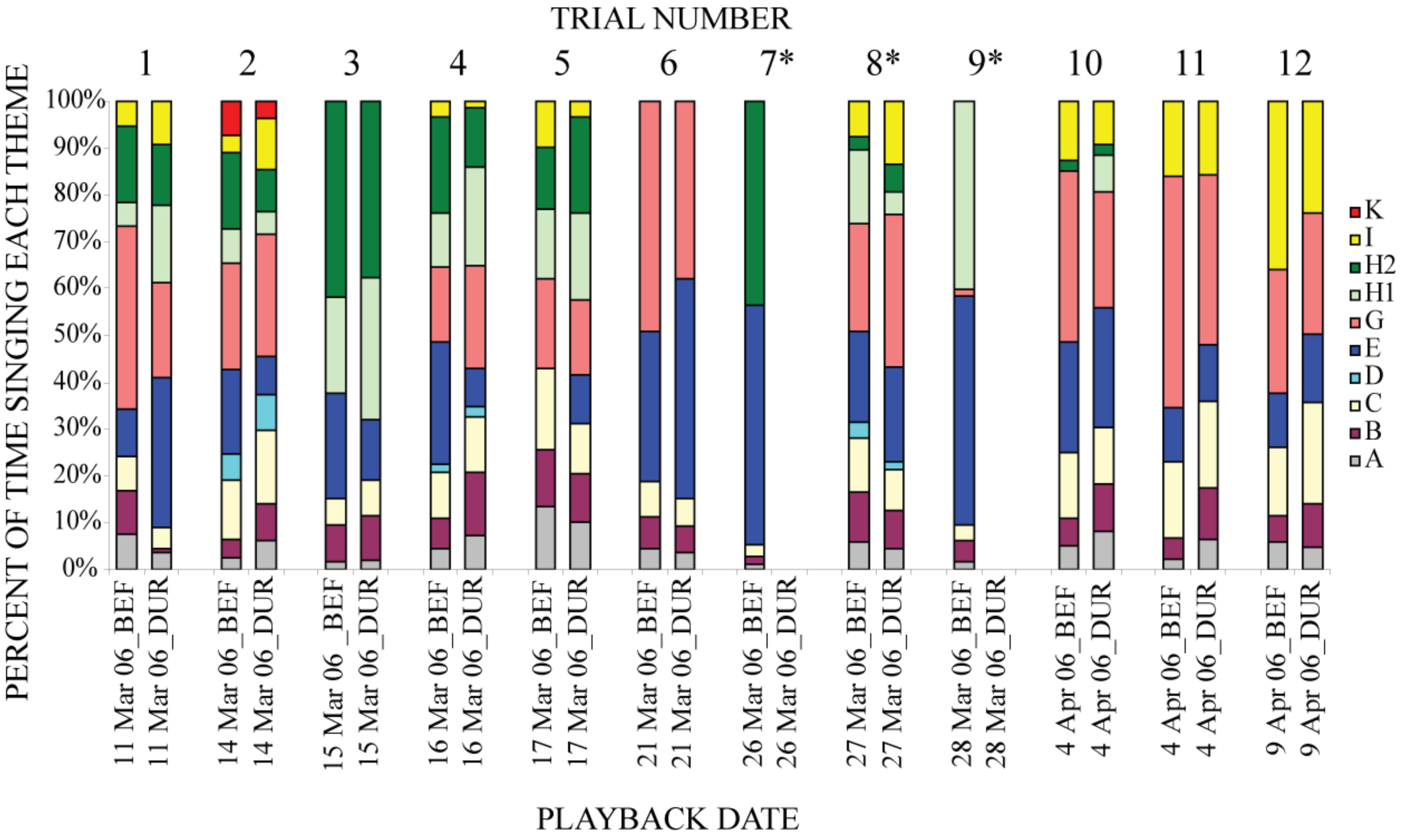
Table 4.1. Outcome summary for twelve song playbacks conducted in 2006. In three trials, the focal singer quit singing during the playback (Q), in all other cases the focal animal continued to sing throughout the playback (C).

Trial #	Date – 2006	Outcome	Latency to Quit (minutes)
1	11 March	C	>45
2	14 March	C	>45
3	15 March	C	>45
4	16 March	C	>45
5	17 March	C	>45
6	21 March	C	>45
7	26 March	**Q**	**1**
8	27 March	**Q**	**37**
9	28 March	**Q**	**9.6**
10	4 April am	C	>45
11	4 April pm	C	>45
12	9 April	C	>45

Song Composition

A total of 10 different themes were identified in the 2006 songs. Only one singer sang all 10 themes (and the 10th theme was particular to this individual, as no other whale sang it). All other males sang between 5 – 9 themes during the analysis period (average = 7 ± 1.7 themes, Figure 4.3).

Figure 4.3: Song composition for each singer represented as the proportion of time spent singing each theme before and during playback experiments. *Three singers quit singing during the playback. The singer in trial #8 did not quit until 37 minutes into the playback; therefore, his song composition both before and during the playback is shown.


TRIAL NUMBER
1
2
3
4
5
6
7*
8*
9*
10
11
12
PERCENT OF TIME SINGING EACH THEME
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
11 Mar 06_BEF
11 Mar 06_DUR
14 Mar 06_BEF
14 Mar 06_DUR
15 Mar 06 _BEF
15 Mar 06_DUR
16 Mar 06_BEF
16 Mar 06_DUR
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27 Mar 06_BEF
27 Mar 06_DUR
28 Mar 06_BEF
28 Mar 06_DUR
4 Apr 06_BEF
4 Apr 06_DUR
4 Apr 06_BEF
4 Apr 06_DUR
9 Apr 06_BEF
9 Apr 06_DUR
PLAYBACK DATE
🟥 K
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🟥 G
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🟦 D
🟨 C
🟪 B
⬜ A

Switching Rate and Versatility

The mean switching rate for the nine singers in the BEFORE period was 0.14 ± 0.05, and their mean switching rate in the DURING period was 0.16 ± 0.04. Thus, there was a trend towards an increase in switching rate, but it was not significant (paired t-test, n=9, t-Ratio: 1.56, p=0.077, Figure 4.4a).

The mean song evenness measure for all singers was 0.79 ± 0.10 in the BEFORE period, and 0.87 ± 0.06 in the DURING period. The increase in evenness was significant (paired t-test, n=9, t-Ratio: 2.25, p=0.03, Figure 4.4b). A test for unequal variances revealed that the variance around the mean significantly decreased during the playback period, suggesting that singers responded similarly to the playback regardless of their level of pre-playback song sequence homogeneity (F-ratio: 5.64, p= 0.03). A non-parametric test performed on the paired singer versatility data was therefore performed and also yielded a significant result (Wilcoxon Signed-Rank test, p= 0.024)

Proportional Representation of Playback Themes

The three themes used for the playback stimuli comprised 24% - 88% of the focal singers' pre-playback song sequences (average = 58 ± 24.7%) (Table 4.2). The same themes comprised 35% - 82% of the singers' sequences during the playback (average = 53 ± 15.5%). The change in proportion of the "playback themes" was not significant (paired t-test, n = 9, t-Ratio = -0.248, p = 0.81). However, a closer examination of the data revealed an interesting trend: the four singers whose playback themes comprised more than 60% of their song sequence in the BEFORE period all decreased the proportion of these themes during the playback; all other singers increased the proportion of these themes (Figure 4.5).

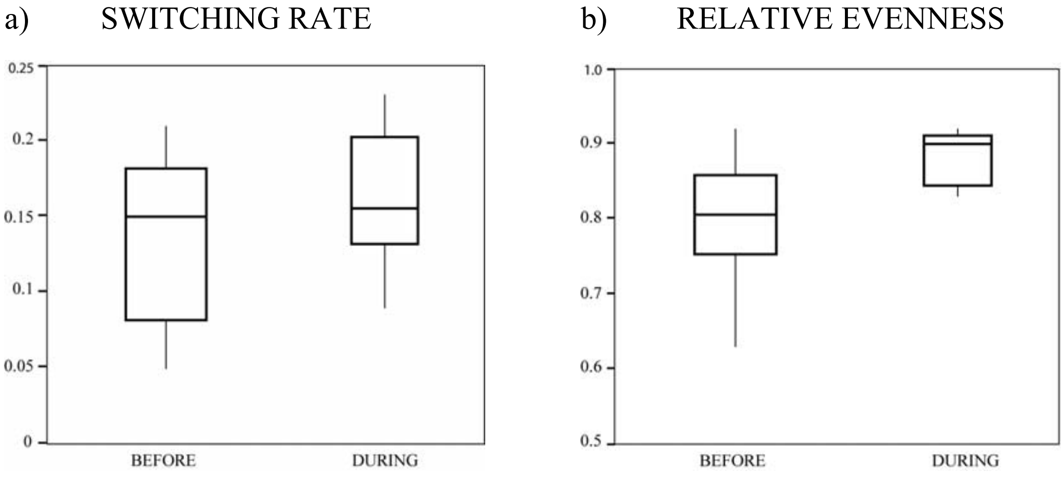


Figure 4.4. Boxplots showing switching rate (a) and relative song evenness (b) both before and during the playback period (n = 9 singers). Arcsine transformed data were tested with matched pairs t-tests; switching rates increased from 0.14 ± 0.05 to 0.16 ± 0.04, but this was not significant (matched pairs t-test, n = 9, t-Ratio: 1.56, p=0.077). Relative song evenness increased from 0.79 ± 0.10 to 0.87 ± 0.06, this difference was significant (matched pairs t-test, n = 9 t-Ratio: 2.25, p=0.03). Additionally, the variance in evenness decreased significantly during the playback period (p = 0.03).

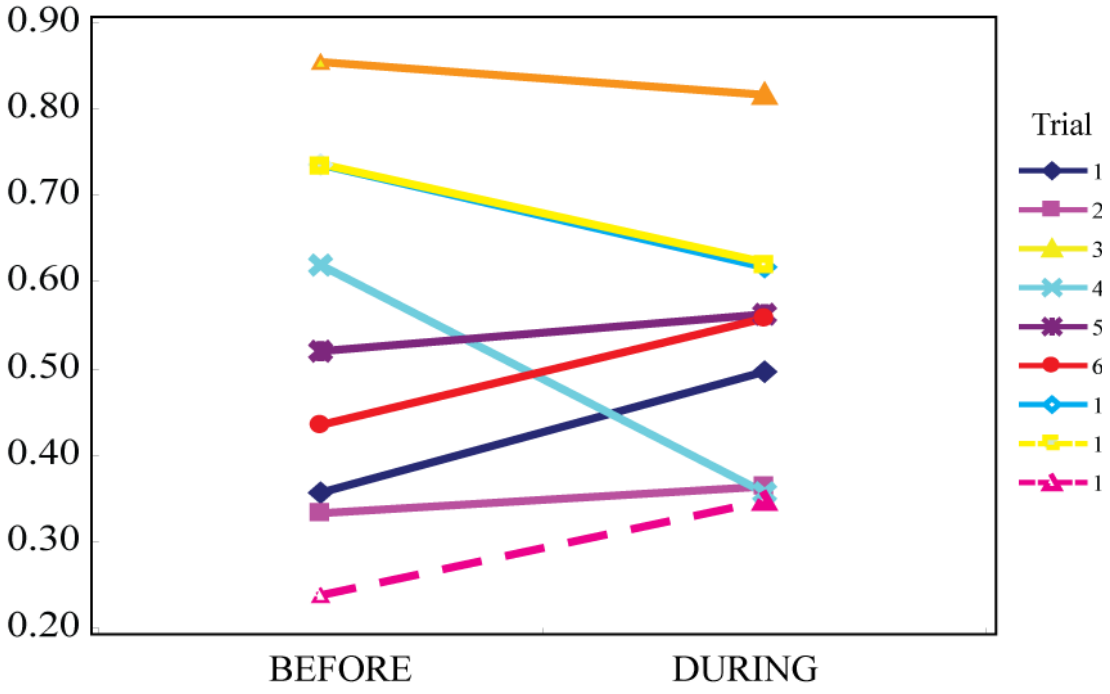


Figure 4.5. Proportion of each singer's song sequence comprised of the "playback themes" in the BEFORE and DURING periods. Only those singers who continued to sing throughout the experiment are shown (n = 9). While the mean proportion before (58 ± 24.7%) and during (53 ± 15.5%) the playback were not significantly different, note that in all cases for which the playback themes comprised 60% or more of the singers' pre-playback song sequences, the representation of these same themes decreased during the experiment (n = 4).

Table 4.2. The proportion of each male's song sequence comprised of the three themes used in their playback trial, calculated for the 45-minute pre-playback period (BEFORE) and the 45-minute playback period (DURING). These proportions were summed to calculate the overall representation of the playback themes in each male's song for the two periods. Note that all four males whose songs were composed of 60% or more of the playback themes decreased their representation of these themes during the playback experiment. In two trials (6 & 12), what were originally considered two different themes were grouped into one for analyses.

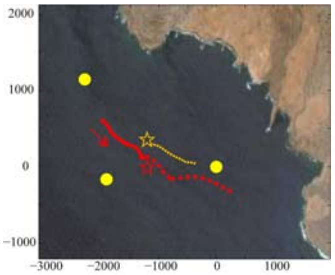
	BEFORE			**DURING**			**OVERALL**		
Trial	Theme 1	Theme 2	Theme 3	Theme 1	Theme 2	Theme 3	BEFORE sum	DURING sum	CHANGE
1	0.10	0.07	0.18	0.32	0.04	0.14	0.36	0.50	0.14
2	0.13	0.13	0.07	0.10	0.12	0.15	0.33	0.36	0.03
3	0.22	0.42	0.22	0.13	0.38	0.31	0.86	0.82	-0.04
4	0.29	0.10	0.23	0.09	0.12	0.15	0.62	0.36	-0.26
5	0.19	0.16	0.17	0.23	0.23	0.11	0.52	0.56	0.04
6	0.36	0.07		0.50	0.06		0.43	0.56	0.13
10	0.23	0.14	0.36	0.25	0.12	0.25	0.74	0.62	-0.12
11	0.12	0.14	0.47	0.12	0.15	0.34	0.73	0.62	-0.11
12	0.12	0.12		0.16	0.19		0.24	0.35	0.11

Spatial Analyses

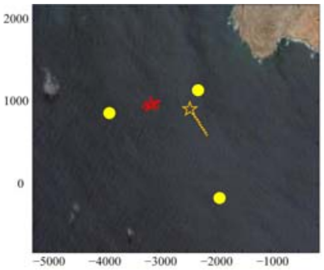
In all cases, focal singers could be acoustically tracked and located for the entire pre-playback and playback period (except in the 3 trials where focal males ceased singing). The playback vessel was acoustically tracked and located in 10 trials; for the other trials, boat locations were obtained using GPS and/or theodolite data (Figure 4.6).

Figure 4.6. Tracks of focal singing humpback whales (red) and playback vessel (gold) generated from acoustic locations calculated every 2.5 minutes or more. Pop-ups are indicated by yellow circles. Note that the scale on each graph is adjusted to show track details, so differs depending on the magnitude of singer movement. Focal singer tracks (red) are divided into BEFORE playback (solid line) and DURING playback (dotted line). The double-headed arrow shows the position and direction of focal singer travel at beginning of analysis period. The red star indicates focal singer location at initiation of playback experiment. Playback vessel tracks (gold) are indicated with a dotted line; gold stars indicate the position of the vessel at initiation of playback experiment. The vessel engine was turned off in all cases, so any vessel movement was the result of drift due to wind and/or current.

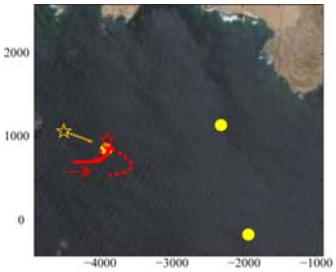
[‡]Singers ceased singing during the playback in three instances (Table 4.1). Since acoustic locations can only be calculated while the individual is singing, the tracks of these males are necessarily truncated.



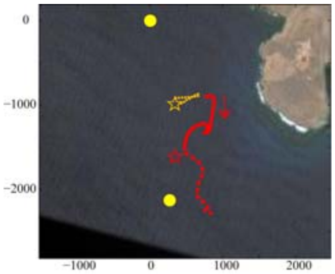
11 March 2006; Trial 1



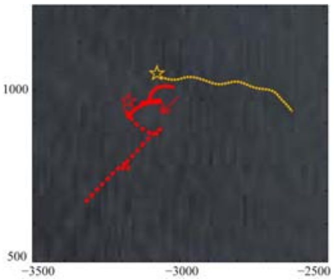
14 March 2006; Trial 2



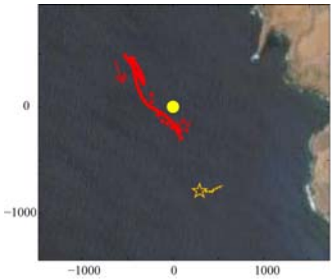
15 March 2006; Trial 3



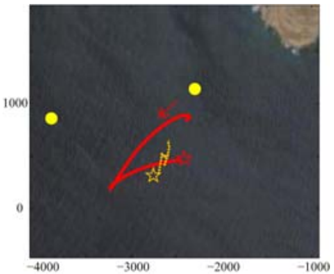
16 March 2007; Trial 4



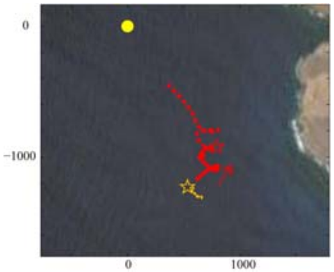
17 March 2006; Trial 5



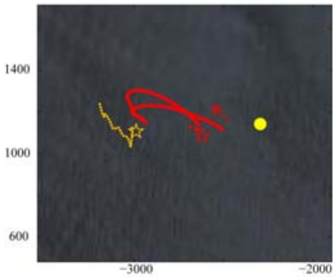
21 March 2006; Trial 6



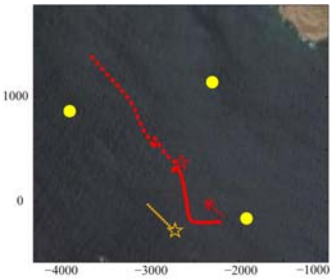
26 March 2006[‡]; Trial 7



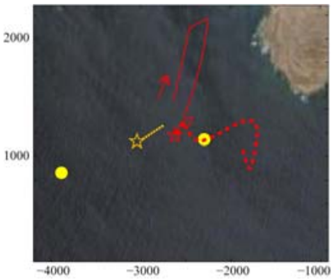
27 March 2006[‡]; Trial 8



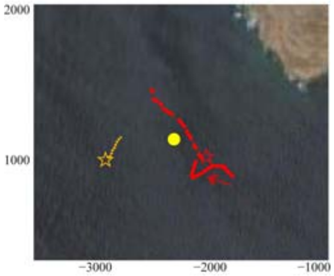
28 March 2006[‡]; Trial 9



4 April 2006 am; Trial 10



4 April 2006 pm; Trial 11



9 April 2006; Trial 12

Meander Ratio

The average meander ratio for all singers was 1.97 ± 0.69 in the BEFORE period, and 1.52 ± 0.55 in the DURING period (Figure 4.7). The increase in directionality was not significant (paired t-test, n = 9, t-Ratio: -1.54, p= 0.16). However, the focal singer on 14 March was essentially stationary (Figure 4.6), if he is removed from the analysis, the increase in directionality for the remaining singers becomes significant (paired t-test, n=8, t-Ratio: -2.58, p=0.04).

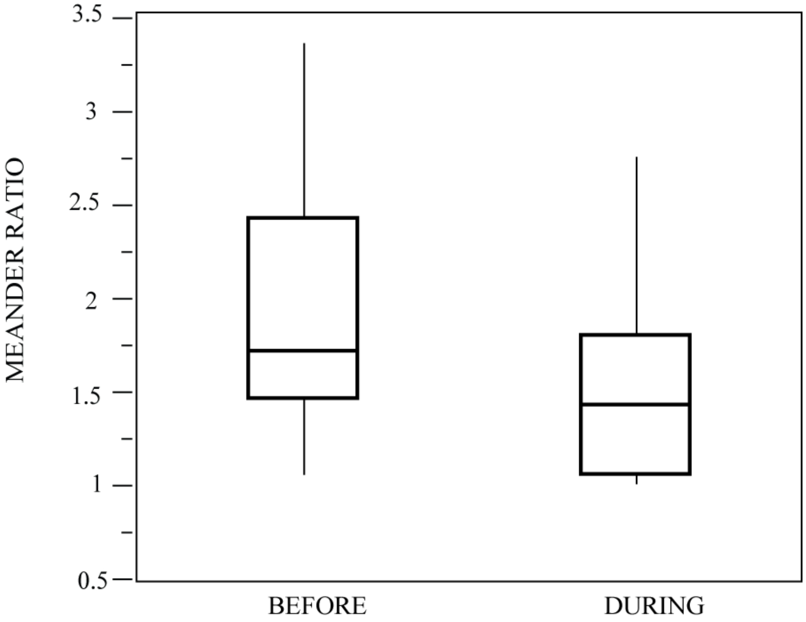


Figure 4.7. Boxplot of focal singer meander ratio before and during playback experiments. Average meander ratio was 1.97 ± 0.69 in the pre-playback period and 1.52 ± 0.55 during the playbacks. This difference was not significant (paired t-test, n=9, t-Ratio: -1.54, p= 0.16). However, when the stationary singer from 14 March 2006 is removed from the analysis, the increase in directionality for the remaining singers is significant (paired t-test, n=8, t-Ratio: -2.58, p=0.04).

Separation Distances

The distance between the focal singer and the playback vessel at the initiation of the playback ranged from 284-742m (average 518 ± 204m, n = 12 trials, Figure

4.8). Ending distances ranged from 310-1500m (average 835 ± 418m, n = 11 trials, not including trial 7 where the singer quit immediately). Overall, singers increased their distance from the playback vessel (mean change in distance was 307 ± 379m, n = 11 trials). However, when examined individually, three singers actually decreased their distance to the vessel over the course of the playback (Trials 3, 9, 12), while eight increased their distance. In trial 9, the singer moved 25m closer to the vessel before ceasing to sing after 9.6 minutes (at a final distance of 367m from the vessel). Additionally, of the eight singers that ultimately moved away from the playback vessel, one of those (Trial 5) appeared to travel generally parallel to the vessel for approximately 20 minutes before clearly moving away.

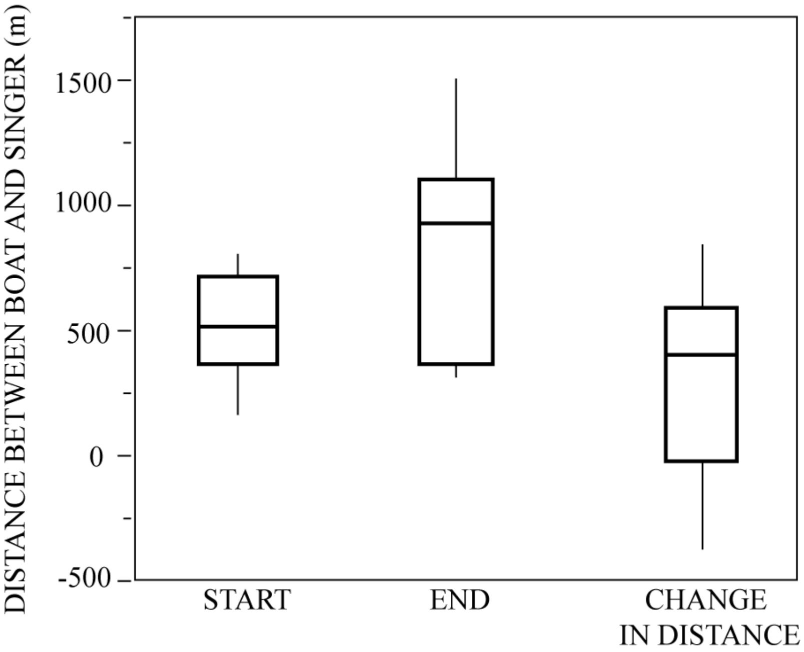


Figure 4.8. Boxplots showing the distance between the focal singer and the playback vessel at the start and end of the playback period (n= 12 and 11, respectively; trial 7 is not included in the ending distance measurements since he quit immediately), as well as the change in distance between the singer and vessel during the playback (n=11). Three singers moved closer to the playback vessel (Trial 3, -189m; Trial 9, -25m; Trial 12, -377m). All other singers increased their distance from the vessel over the playback (mean change over all trials = 307 ± 379m).

The change in distance between the focal singers and the playback vessel was compared to the mean of the null distribution of change in distance generated from pairs of non-interacting singers (see Chapter 3, mean change in distance = 362m). The observed change in distance during the playback trials was not significantly different from the null distribution (Wilcoxon Sign-Rank, p=0.88).

DISCUSSION

The results of this playback experiment reveal a different and greater response to the presentation of humpback song than previously noted in the literature. In general, singers altered their acoustic behavior in response to the playback stimulus presentation, suggesting that they are indeed listening to other singers around them. They significantly increased the overall evenness of their song sequences during the playback trials, which resulted in a less repetitive, more "diverse" song presentation. Similarly, there was a trend towards an increase in their switching rate during playback trials. If they sang "typical" song cycles (see Chapter 1), then this would result in "shorter" songs, by the traditional measures used for analyzing humpback song sequences.

The spatial data were somewhat more equivocal, as three of the eleven singers (not including the one who stopped singing immediately) moved closer to the vessel, while eight increased their distance from the vessel. Of the eight that increased their distance from the vessel, one singer (Trial 5) appeared to initially travel somewhat parallel to the playback (even decreasing his distance slightly), before changing direction and clearly moving away. Another singer (Trial 1), traveled parallel to the vessel at a distance of approximately 300m before moving ahead and increasing the distance between himself and the vessel. Taken as a whole, the change in distances were not different from what one would predict based on a null model generated from non-interacting (i.e., non-simultaneous) singers. This suggests that there was not a clear avoidance response to playback, as has been noted in previous studies (Tyack et al. 1983). Additionally, at least two singers ceased singing and approached the playback vessel to within 50m. While this cannot necessarily be interpreted as an aggressive response, it does indicate awareness and the ability on the part of the singers to detect and locate the playback vessel.

The meander ratio data suggest that, overall, singers did not significantly alter the directionality of their travel in response to the playback experiments, although there was a trend towards an increase in directionality during the playback.

Only two playback experiments have been previously conducted to humpback whales in more than an anecdotal way to investigate the response of humpback whales to a variety of conspecific sounds (and presumably to investigate the function of humpback song within the breeding system). In the first of these, Tyack et al. (1983) presented six singers with paired stimuli consisting of humpback song and social vocalizations. These authors found that 2 of the 3 singers that were presented first with song stopped singing in response to the playback. Of 16 total groups (including these six singers and other groups) who were presented with song, 14 of them moved away during the playback. The authors concluded that humpback song did not act as an “attractant”, and that in general, individuals avoided singing males.

In contrast, a subsequent study published by Mobley et al. (1988) found that, of 47 pods that were presented with humpback song stimuli, there was no overall avoidance response. Three pods rapidly approached the vessel during song playback, although two of these were in response to presentation of outdated song (recorded in previous years). Outdated song may therefore have been recognized as unusual in the current context, and the rapid approaches of these two pods may not represent the same functional response as to that of current song. Interestingly, no targeted singers approached the playback vessel during any of the song playback trials.

While the two previous studies attempted to interpret the function of humpback song within the framework of spatial approaches, neither one evaluated the acoustic responses of singing males to the presentation of song playbacks. However, song may be considered a long-range signal, especially within the aquatic environment, and it is reasonable to question whether males are interacting acoustically even in the absence of overt spatial interactions. In fact, an entire body of literature has been developed on assessing the interactions of individuals during song displays (see Todt & Naguib 2000), and many studies have demonstrated the acoustic response of individuals to both natural and experimental stimuli in other species (e.g., Falls et al.; 1988, Nielsen & Vehrencamp 1995; McComb et al. 2003).

Playback studies with territorial songbirds have successfully demonstrated the function of song to mediate both inter-sexual as well as intra-sexual interactions between individuals. For example, it has been shown that males of some species distinguish acoustically between neighbors and strangers (Molles & Vehrencamp 2001), that males use song parameters to indicate an escalation of aggression (McGregor et al. 1992), and that males may use different songs in different social contexts (Catchpole & Slater 1995). Moreover, females of some species may use song characteristics for choosing potential mates (e.g., repertoire size, Searcy & Yasukawa 1996), and individuals may eavesdrop on the interactions between two singing males and use information gained from those encounters to inform subsequent decisions (Naguib et al. 1999).

The interpretation and subsequent evaluation of the function of song within the humpback breeding system is more complicated than that of terrestrial animals. First, humpbacks are not territorial in the traditional sense, which makes it extremely challenging to evaluate whether males are using song to mediate intra-sexual interactions. Not all males may be motivated to interact with one another, and variables that are intrinsically impossible for researchers to evaluate (e.g., age, male quality, experience, familiarity with potential competitors) may play a role in determining whether any two particular singing individuals will engage in an acoustic interaction. These variables cannot be manipulated or even measured in most cases, thereby limiting our ability to undertake such experiments as neighbor-stranger or age-stratified playbacks. In this study, we tried to control for many of these variables by presenting each male with a modified sequence of his own song in order to minimize the effects of perceived differences in quality, etc. Second, although many types of behavior may be observed during surfacing intervals, some of which may be clearly interpreted as aggressive responses (e.g., head lunging), we understand very little as to what types of interactions may occur between individual humpbacks while underwater, or how to interpret them. Therefore, although two singers clearly approached the playback vessel in this study, we cannot with confidence interpret whether or not this should be considered an aggressive response (in contrast to playbacks with songbirds who may become clearly agitated and/or physically attack the playback speaker). Finally, because females are largely not vocal during the breeding season (as far as we know), observing and tracking them for long periods of time is exceptionally difficult. Therefore, we do not know how females respond to singing males, or how they may respond to a playback of male song such as in the current study.

The current study does not fully answer the question regarding the function of song in humpback whales. It does, however, provide evidence that singing males are altering their song presentation in response to the playback of a song stimulus, and are responding acoustically similarly to the way in which they respond to the presence of additional singers (Chapter 3). In almost all cases males responded by increasing the evenness of their song sequence (or decreasing their relative repetitiveness), but more detailed evaluation of potential song matching is required to determine whether these focal singers may have been directing their song towards the playback "intruder". The sample size of this study precludes analyses that combine acoustic and spatial responses that might shed light on how males could be using song to mediate potential displacement or competition. However, the advantage to the methodology employed in this study is that singing males were continuously acoustically located and tracked, which provides much more information regarding their movements relative to the playback vessel than in previous studies (where singers could only be located when they surfaced, which may only happen once or twice during an entire playback trial). Further work along these lines may provide enough data to more clearly evaluate under what circumstances singers choose to approach vs. avoid the playback vessel, and combine that information with their acoustic responses. In summary, the results of this study demonstrate that playback experiments to singing humpback whales have great potential for revealing and evaluating the function of song within this species.

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