UNIVERSITY OF CALIFORNIA, SAN DIEGO

Characterizing population structure of cetaceans within an ecological context

A dissertation submitted in partial satisfaction of the requirements for the degree
Doctor of Philosophy

in

Oceanography

by

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2013

The Dissertation of Alyson H. Fleming is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

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2013

DEDICATION

For my Dad, the greatest professor and father I have ever known. I will carry the spider stick with pride.

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Chapter 2, in part, is a reprint of the previously published material as it appears in NOAA-TM-NMFS-SWFSC-474, 2011, Fleming, AH and Jackson, J. The dissertation author was the primary investigator and author of this paper.

Chapter 3, in full, will be prepared for submission for publication of the material. Fleming, AH, Barlow, J, Calambokidis, J. The dissertation author was the primary investigator and author of this paper.

Chapter 5, in full, is currently being prepared for submission for publication of the material. Fleming, AH, Yack, T, Barlow, J. The dissertation author was the primary investigator and author of this paper.

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ABSTRACT OF THE DISSERTATION

Characterizing population structure of cetaceans within an ecological context

by

Alyson H. Fleming

Doctor of Philosophy in Oceanography

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Professor Jay Barlow, Chair
Professor Lisa Levin, Co-chair

Detection of population structure is critical to the management and conservation of wildlife populations. Cetacean populations are protected under US law, necessitating accurate information on population structure, yet identification of such structure is inherently difficult due to the highly pelagic and mobile nature of most cetacean species. The factors that lead to population divergence and eventual speciation are complex, and current cetacean population structure is the result of both evolutionary and ecological processes. This dissertation examines potential ecological mechanisms of divergence in order to improve detection of differences among conspecific populations.

To understand the importance of population structure information in establishing marine mammal policy priorities and management plans, I examined both the policy process and the scientific data utilized in a global review of humpback whales under the US Endangered Species Act (ESA). The challenges in this review process highlighted the importance of population structure information and the utility of multiple lines of evidence in resolving structure at both demographic and evolutionary scales.

Foraging ecology and prey selectivity may be possible drivers of ecological divergence between cetacean populations. Using stable isotope analysis, I examined the diet consistency of a single population of humpback whales in the California Current over decadal time scales. Diet varied significantly over the study period, suggesting that this population of humpback whales shows a high degree of foraging plasticity and that diet may not be a consistent marker of population identity for this species.

Since future investigations of cetacean population foraging ecology and structure based on stable isotope methods require understanding of individual isotopic variability, I quantified indiviudal variability in humpback whale tissue due to physiological processes and tissue preservation methods and found that individual variability was less than that associated with a trophic level shift and is not prohibitive for investigations of trophic differentiation in cetaceans.

Lastly, since habitat specialization may also drive ecological divergence, the novel application of passive acoustics enabled me to better characterize and predict the habitat preferences of a poorly-described ceteacean species, Dall's porpoise. This predictive understanding allows for better estimation of population distribution, abundance and structure.

Chapter 1.

Introduction

The identification and classification of biodiversity has been at the heart of biological research even before Linnaeus proposed his hierarchical system for taxonomic classification in the 1730s. This ordering and cataloguing of evolutionary units has enabled scientists to decipher process and function in the collection of life on earth. Taxonomic focus has largely been at the species level (Agapow *et al.*, 2004). Despite much debate surrounding the species concept, a species designation represents biological, ecological and evolutionary progress – stepping stones that both scientists and policy-makers have determined to be worthy of preservation (Agapow *et al.*, 2004; Taylor, 2005). However, definable units exist even below the species level, subspecies and demographically independent populations, which show divergence from neighboring populations (Taylor, 1997; Taylor, 2005; Clapham *et al*, 2008). One of the explicit goals of marine mammal management is to preserve not only the diversity of species and subspecies that exist today as functioning elements of the ecosystem but also the evolutionary potential of a population (Taylor, 1997). In the near-term, divergence between populations has ramifications for effective conservation and management, especially if the populations face variable levels of threats and inter-population dispersal rates are too low to sustain the impacted population (Taylor and Dizon, 1999). In the long-term, divergence between these populations may place them on distinct evolutionary trajectories that will lead to the creation of separate species (Taylor, 2005).

Within both the Endangered Species Act (ESA) and the Marine Mammal Protection Act (MMPA), units below the species level ("stocks" under the MMPA and

"distinct population segments" under the ESA) have been granted recognition as conservation-worthy targets. Of course, the listing of these units depends upon the scientific identification of them. Since these units are inherently less divergent, the same phylogenetic tools that allow for detection of species-level differences can't always decipher populations that may be demographically, though not yet genetically, distinct. Phylogenetic analyses typically infer, retrospectively, a divergent process that has long since resulted in the splitting of groups into species (Wolf *et al.,* 2008). So what markers may be used for classifications at the subspecies and population level? Essentially, what tool will provide an indication of early steps of divergence?

Considering the causal mechanisms that eventually lead to cetacean speciation may inform identification of population divergence (Schluter, 2001;Wolf *et al.,* 2008). The marine environment is theorized to be a high gene flow system especially for top predators with vast dispersal opportunity across large ecosystem ranges. For cetaceans, ranges of individuals and populations can be thousands of miles in a single season (Mate *et al.,* 1999; Weise *et al*., 2006). While reproductive isolation at distances greater than individual ranges is expected through geographic isolation, sympatric divergence has been observed across much smaller scales and across environmental gradients without apparent geographic boundaries (Wolf *et al.,* 2008; Fontaine *et al.,* 2007). This suggests that ecological processes may be a possible contributor to selective divergence.

Ecological speciation results from the development of reproductive isolation due to divergent adaptation to environmental conditions (Schluter, 2001; Rundle & Nosil, 2005). Causes of such selection can be biotic, abiotic or mediated by conspecific or interspecific interactions (Schluter, 2001; Rundle & Nosil, 2005). These factors may lead

to trophic discrepancies between two populations in the form of resource partitioning and niche segregation (Hoelzel *et al.,* 2007). Alternatively, habitat specialization may drive population separation (Bierne *et al.,* 2003). Habitat specialization may result from competition for space, food, or other resources, physiological constraints or novel ecological opportunities (Schluter, 2001;Wolf *et al.,* 2008). Also, the composition of other species in the local environment may create selective pressure on mating and social signals such as morphology, body size, communication calls, and/or coloration patterns (Schluter, 2001). In cetaceans and other social mammals, learning and social structure may be another force interacting with these ecological causes of divergence. Habitat preference and foraging behavior is often learned behavior in many species of cetaceans (Beltman & Haccou, 2005; Slagsvold & Wiebe, 2007). Young of some species stay in direct contact with their mother for the first year of life and will return to the natal mating and feeding grounds annually (Martin *et al.,* 1984; Baker *et al.,* 1990). Cooperative feeding strategies are also usually learned behaviors that may reduce incentives to dispersal (Weinrich, 1991; Ford *et al.,* 1998; Allen *et al.,* 2013). This social component likely also contributes to reduced gene flow and plays a role in the structuring of cetacean populations.

Since ecological factors may create divergence between populations overtime thereby driving speciation, examination of these ecological factors may enable detection of discrete populations (Schluter, 2001;Wolf *et al.,* 2008). Foraging behavior and habitat specialization are two drivers that have already been applied to studies of marine mammal population structure (Natoli *et al.,* 2005; Witteveen *et al.,* 2009). Stable isotope analysis has proven to be effective at resolving spatial or ecological distinctiveness

between populations over short time scales (1-2 years) (Born *et al.,* 2003;Witteveen *et al.,* 2009). Differences in stable isotope signatures can result from both geographic and trophic differences between populations, indicating disparate feeding locations, trophic levels or prey types, suggestive of niche segregation (Farquhar *et al.*, 1989; Post, 2002; Newsome *et al.*, 2007). Habitat specialization has been documented at both small and large spatial scales in both pelagic and coastal ecosystems (Redfern *et al*. 2006; Williams *et al.,* 2009). Habitat specialization in marine mammals and, cetaceans in particular, has largely been gleaned through observations, tagging data, and acoustic presence/absence data that provide relatively short temporal windows into habitat preferences and specialization (Fiedler *et al.,* 1998; Friedlaender *et al.,* 2011; Elliott *et al.,* 2011).

Trophic and habitat-related lines of evidence have proven useful in the identification of some populations and are especially constructive as complements to morphological or genetic data (Taylor, 2005; Wolf *et al.,* 2008). However, there is substantial uncertainty regarding population-level temporospatial variability in basic ecological characteristics such as prey selection, foraging location and habitat specialization. Therefore, for rigorous application and interpretation of ecological divergence between populations, consistency of ecological characteristics within a population must first be determined. This within-population information has been absent from most studies to date that have applied measures of ecological divergence to investigations of population structure. To address this common deficiency, my dissertation examines temporospatial variability in cetacean population foraging ecology and habitat preference. This should provide new insight on the ecological adaptability of

cetacean populations in light of changing oceanographic conditions, prey availability, and habitat quality and improve detection of discrete populations.

Ignorance of cetacean population structure significantly impairs management efforts by complicating attempts to meaningfully estimate abundance, detect population trends and assess demographics (Taylor & Dizon, 1999). The second chapter of this thesis examines the critical role that population structure plays in establishing marine mammal policy priorities and management plans. Using humpback whales (*Megaptera novaeangliae)* as a case study for the interpretation of population structure data within a management context, I present a summary of a recent global review of the species that was undertaken by the National Marine Fisheries Service to evaluate the current listing of the species under the ESA. A major focus of this status review was a revision of the population structure of the species, as listed under the ESA, from a single global species to numerous DPSs. The data that were relevant to the listing determination of the North Pacific humpback whale populations are summarized and the identified distinct population segments are presented. With many marine mammals showing positive abundance trends, and some nearing pre-exploitation levels, such revision under the ESA may become more frequent in the near-future, making this review very relevant to the management of protected species.

Since foraging ecology and prey selectivity may be drivers of population divergence, the third chapter of this study examines temporal variability in the diet of humpback whales in a single feeding population in the California Current ecosystem of the eastern North Pacific. Observations in the field suggest that this species is a largely opportunistic forager and may switch prey bases depending on relative prey availability

in the system. However, humpback whale prey selection over multi-year scales had never been investigated. This study utilizes stable isotope ratios of carbon and nitrogen to assess trophic consistency over two decades. This work provides baseline information on population-level variability with implications for the use of stable isotope ratios in the determination of population differentiation.

Recognizing that long-term studies may reveal important ecological insights, the fourth chapter of this study examines some of the methodological issues involved in conducting multiyear trophic studies with stable isotope analysis. Sample preservation methods often change over time, introducing potential compounding factors to the interpretation of isotope ratios and their ecological relevance. Additionally, variation in both the diet and physiology of individuals may impact observed stable isotope ratios. Assessing this individual variability before interpreting population-level patterns is as necessary a step as evaluating population-level variability before interpreting patterns between populations.

Lastly, since habitat preferences and specialization may also be a driving force of ecological speciation, for my fifth chapter I built habitat models for Dall's porpoise (*Phocoenoides dalli*), a widely-distributed small odontocete with poorly described population structure. Acoustic detections of the species were used to model the encounter rate of the species in response to a suite of static and dynamic environmental variables. This work represents the first application of acoustics to habitat modeling for this species and one of the first for any species. By utilizing acoustic detections, we gain a more thorough view of population distribution and habitat preference than is gleaned from surface-observations alone.

Each of the following chapters is intended to stand alone as a publishable unit and the reader may find some repetition in the introduction of each chapter. Chapter 2 is partially adapted from another publication that I co-authored, entitled “Global Review of Humpback Whales (*Megaptera novaeangliae*)” and is presented as part of this dissertation with permission from my co-author (Fleming & Jackson, 2011).

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Chapter 2.

North Pacific humpback whale population structure and status under the Endangered Species Act

Abstract

Humpback whales (*Megaptera novaeangliae*) are currently classified as Endangered under the U.S. Endangered Species Act (ESA) and are listed as a single species worldwide. However, genetic, photo-ID and telemetry studies have shown that they occur in distinct population segments (DPS) in each ocean. Although humpback populations were reduced to fractions of their original size by commercial whaling, many populations have shown consistent growth over the past few decades. Recent studies estimate abundance in the North Pacific and North Atlantic to be approximately 20,000 and 12,000 respectively. In the Southern Hemisphere, Australian stocks have been growing at a rate of 10-12% per year while other stocks have shown slower growth or even decline. Prompted by this variable pattern of recovery between stocks, the National Marine Fisheries Service initiated a worldwide review of the species under the ESA, evaluating the biological justifications and status of each population. This chapter provides a review of both the ESA assessment process and the information that was utilized in the ESA assessment to determine DPSs in the North Pacific.

Introduction

Natural resource management depends on the classification of species into discrete population units. This requires both taxonomic-level classification (species and subspecies) and the identification of distinct populations within a species. Though taxonomic classifications are often viewed as fixed identifiers, taxonomy is, in fact, a

dynamic process that must continuously decipher and revise evolutionary units. As new data come to light, taxonomy is often revisited and the very definition of species, subspecies and lower levels of classification is the subject of ongoing debate (Clapham *et al*., 2008; Wade & Angliss, 1997; Taylor & Dizon, 1999; Taylor 2005). While deciphering species-level classifications can be complex, identifying the smaller differences between populations within a species poses even greater challenges. Morphological and genetic data provide support for species determinations but the detection of demographically independent populations often requires multiple lines of evidence, each of which reveals more subtle divisions between neighboring groups than those seen at the species level. Although these subtle demographic differences are hard to identify, they are the most relevant to managers since the maintenance of a species optimal abundance and complete geographic range requires management actions to be targeted at the population level.

These difficulties involved in identifying conservation units complicate the management and conservation of cetaceans. Cetaceans pose a particularly unique set of challenges in the definition and identification of conservation units because many cetacean species are globally distributed and can migrate across entire ocean basins. In addition, their population structure is difficult to discern due to complex and variable life-history patterns and habitat boundaries in the marine environment that are difficult for us to recognize. Finally, obtaining samples to refine our understanding of these dynamics is often difficult and costly.

Marine mammal conservation, and cetacean conservation especially, is also highly politicized, particularly in the United States. All marine mammals are afforded

special protection under the Marine Mammal Protection Act (MMPA) which requires that populations maintain abundances over 50% of historical levels and the full geographic range of the species. Ten of the large whale species are also listed as Endangered under the Endangered Species Act (ESA). Numerous non-governmental organizations lobby and litigate on behalf of marine mammals and at least $25 million is spent annually on whale conservation by these NGOs (Costello *et al*., 2012). This level of political and social and economic investment in cetaceans means that the selection of conservation units is far from a purely scientific endeavor.

The structure of the MMPA and ESA reflect the history of negotiations between cetacean research and management groups. Both laws typically manage a species for conservation on a scale below the species level, reflecting a shared priority to conserve species as more than a collection of remnant populations. Under the ESA, conservation efforts below the species level are often focused on distinct population segments (DPSs). A DPS is determined by 3 criteria: (1) the discreteness of the population from the rest of the species, (2) the significance of the population to the species and (3) the endangerment status of the population. Discreteness may be established by physical, physiological, ecological or by behavioral differences or separation by international boundaries. Significance may be established if the population persists in a unique setting, if the loss of that segment would cause a gap in the species range, if the population shows genetic differentiation or if it represents the only remaining natural occurrence of the species. Despite these explicit criteria, identifying DPSs in practice and determining their conservation status is an iterative and challenging process due to uncertainty in assessing the ESA status of cetacean populations and identifying conservation units (Fig. 2.1).

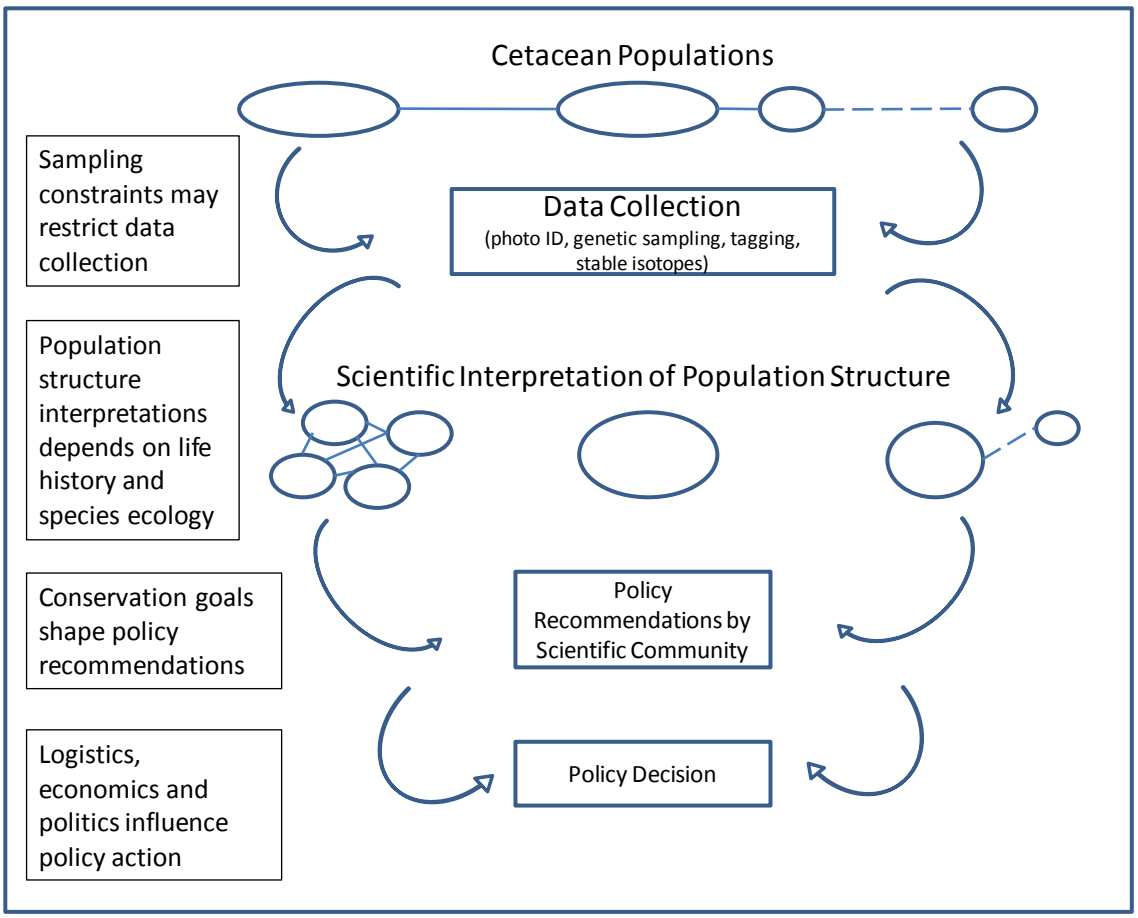

Fig. 2.1: Flow chart outlining the process of determining distinct population segments (DPSs) under the U.S. Endangered Species Act.

Humpback whales, *Megaptera novaeangliae*, are globally distributed baleen whales with long pectoral flippers, distinct ventral fluke patterning, dark dorsal coloration, a highly varied acoustic call and a diverse repertoire of behavior (Clapham & Mead, 1999). In December 1970, the humpback whale was listed as endangered under the Endangered Species Conservation Act of 1969. When the Endangered Species Act (ESA) was passed in 1973, the humpback whale was automatically incorporated onto the ESA's List of Endangered and Threatened Wildlife and Plants with an endangered designation, along with all other previously listed large whale species. Since the original Act did not allow for DPSs, all humpback whale populations were listed as one global entity or

species under the Act. Since then, decades of research has revealed that humpback whales have a complex population structure and that a single global ESA status is inappropriate. Recent evidence of marked population growth prompted a review of the ESA listing status of humpback whales by the National Marine Fisheries Service. A Biological Review Team of experts was assembled in order to evaluate all current scientific information on the species and make a policy recommendation detailing the structure and number of DPSs and the extinction risk of each one. Ultimately, the final ESA listing revision will be a decision at the policy level but it will be based on the BRT's assessments. It is important to note that the BRT can't make direct recommendations of endangerment status, but instead must conclude with an evaluation of extinction risk.

The global distribution and complex population structure of humpback whales create an excellent case study for examining the process, data needs, and challenges involved in the management and conservation of migratory species. Though humpback whales around the world share many characteristics, distinctions do exist. Identification of discrepancies between segments of the global population involved both subspecies and DPS determinations. The distribution of every DPS spans multiple countries. While some regions face considerable threats, most have experienced remarkable population growth and recovery.

This chapter presents a review of the most relevant information for assessing the ESA status of North Pacific humpback whales. It was derived from deliberations by the BRT and from a global review of humpback whales that I co-authored in order to provide the BRT with comprehensive information needed for their assessment (Fleming &

Jackson, 2011). The structure of the chapter largely follows the structure of the ESA review. I begin with a review of the information needed to establish discreteness and significance and then discuss data relevant to extinction risk assessment. Since evaluations of discreteness and significance criteria for potential North Pacific DPSs are made with respect to the next highest taxonomic order, the existence of humpback whale subspecies is the first topic presented here and was the first question addressed by the BRT (Section I). To assess discreteness and significance of populations in the North Pacific, information on population structure, distribution, migration and genetic diversity is reviewed (Section II). This section concludes with a summary of the BRT's DPS determinations. Next, I present data on abundance, trends and threats and a summary of the BRT's evaluations of extinction risk (Section III). I close with some brief reflections on the science-policy interface that may be gained from viewing this ESA-listing review process.

I. Distinction between Northern and Southern Hemisphere Humpback Whales

Historically, numerous subspecies of humpback whales were named. They are not widely recognized and *Megaptera noavaeangliae (Borowski, 1781)* has remained the accepted taxonomic classification (Clapham & Mead, 1999). However, the current monotypic species classification of humpback whales was revisited during the BRT deliberations. To help with this portion of the assessment, the Ad-hoc Committee on Taxonomy of the Society for Marine Mammalogy was consulted. The following information was considered in order to determine whether the humpback whales that feed

in the North Atlantic, North Pacific, Southern Ocean and Arabian Sea likely belong to distinct subspecies.

Individual humpback whales in the Southern Hemisphere differ from those in the two Northern Hemisphere oceans in the patterning and extent of ventral fluke and lateral pigmentation (Rosenbaum *et al.*, 1995), as well as in the timing and location of reproduction. Observations indicate that mating occurs six months apart in the two hemispheres. Differing estimates of testis weight from the breeding and feeding grounds (and no spermatozoa detected on feeding grounds; Symons and Weston, 1958) indicate that there is seasonal variation in sperm production (Chittleborough, 1965; Omura, 1953), further supporting the asynchrony of seasonal mating between the Northern and Southern Hemisphere populations. Ovulation is also seasonal (Chittleborough, 1957), suggesting that if individual whales travel between the hemispheres outside their usual estrus period, this seasonality may prohibit successful reproduction. However, encounters on common breeding grounds between whales at the very end or start of their respective winter breeding seasons e.g. in Panama and Costa Rica, may result in successful reproduction.

In the southeastern Pacific Ocean some southern-summering humpback whales migrate to Northern Hemisphere breeding grounds in waters off Central and South America (e.g. Acevedo and Smultea, 1995; Flórez-González *et al.*, 1998; Rasmussen *et al.*, 2007; Stone *et al.*, 1990), a region which may be frequented by whales from North Pacific Ocean populations during the winter (Acevedo and Smultea, 1995). It is therefore possible that inter-hemispheric migratory movements and/or mating events take place between populations along the Pacific coast, although there is no genetic, satellite telemetry or sightings evidence for this type of exchange (Baker and Medrano-González,

2002). A similar pattern occurs in the southeastern Atlantic, where southern-summering humpback whales have been sighted and stranded in Central West African countries as far north as 6°N, including Benin, Ghana and the eastern Ivory Coast (Van Waerebeek, 2003; Van Waerebeek *et al.*, 2007; Van Waerebeek *et al.*, 2009). The southerly extent of the eastern North Atlantic humpback breeding ground is not well described, although whales found in the Cape Verde Islands (14°N) are geographically distant from the known distribution of Southern Hemisphere humpback whale populations. As in the Pacific, there is no genetic, satellite telemetry or sightings evidence for inter-hemispheric exchange in this region.

Genetically, humpback whales in the three ocean basins cannot be defined as 'evolutionary significant units' or ESUs as based on the criteria of Moritz (1994) because mitochondrial DNA (mtDNA) is not reciprocally monophyletic among the ocean basins, *i.e.*, the genetic lineages in each northern ocean do not share a recent common ancestor and are nested among the Southern Hemisphere lineages. The global pattern of maternally inherited mtDNA indicates the occurrence of more than one historical introgression into each of the Northern Hemisphere ocean basins from the Southern Hemisphere, with multiple Northern Hemisphere clades (closely related mtDNA lineages) nested within the Southern Hemisphere clade (Baker and Medrano-González, 2002; Baker *et al.*, 1993). However present gene flow between the Northern and Southern Hemispheres is very limited, estimated at 1-2 females per generation (Baker and Palumbi, 1997). Such limited gene flow strongly suggests both ecological and evolutionary differentiation under a variety of population differentiation criteria (Waples and Gaggiotti, 2006).

The Taxonomy Committee concluded that if a full taxonomic revision of the species was to be conducted, the North Atlantic, North Pacific and Southern Hemisphere humpback whales would likely be named as three separate subspecies. The Arabian Sea was found to be part of the putative Southern Hemisphere subspecies. Therefore, the BRT conducted all further DPS evaluations with respect to these basin-wide subspecies.

II. Differentiation among North Pacific humpback whale populations

A. Distribution & Population Structure

Humpback whales in the North Pacific undergo seasonal migrations from northern-latitude feeding areas in the summer months to more southern-latitude breeding areas in the winter months. Feeding areas are dispersed across the Pacific Rim from California, USA to Hokaido, Japan. Within these regions, humpback whales have been observed to spend the majority of their time feeding in inland and coastal waters. Much more is known about the humpback whales occurring east of the Aleutian Islands than elsewhere; and the western feeding grounds remain relatively understudied.

Breeding areas in the North Pacific are more geographically separated than the feeding areas and include regions offshore of mainland Central America; mainland, Baja California and the Revillagigedos Islands, Mexico; Hawaii; and Asia including Ogasawara and Okinawa Islands and the Philippines. About half of the humpback whales in the North Pacific Ocean breed and calve in the US territorial waters off Hawaii, and more than half feed in US territorial waters (Calambokidis *et al.,* 2008).

As data gathering, particularly photo-identification and genetic studies, increased from the mid-1990s to the present, distinctions among populations have been refined. An increasing number of relatively distinct groups have been identified, starting with the

separation of western and eastern stocks (Darling *et al.*, 1996; Darling and Cerchio, 1993; Darling and McSweeney, 1985). The eastern stock was then genetically recognized as being made up of two separate groups - a central stock that feeds in Alaska and breeds in Hawaii and an "American" stock that feeds in waters off California and breeds offshore of Mexico (Baker *et al.*, 1994). The "American" stock was then subdivided again, making the Mexico offshore breeding stock (with feeding destination then currently unknown), separate from the continental Mexican stock that migrates to the waters off California, Oregon and Washington States (Barlow, 1994; Barlow *et al.*, 1997).

Between 2004 and 2006, a multinational coordinated study called Structure of Populations, Levels of Abundance and Status of Humpbacks (SPLASH) examined humpback whale population structure and abundance in the North Pacific. Field studies were conducted at all known North Pacific breeding and feeding areas. A total of 18,469 quality fluke identification photographs were taken, producing a total of 7,971 unique individuals cataloged. A total of 6,178 tissue samples were also collected for genetic studies of population structure, with fairly even representation of wintering and feeding areas. With the completion of the project's field components, greater resolution of migratory connections and interchange between and within regional populations has been possible. It is now very clear that a great deal of structural complexity exists within the North Pacific and that it does not contain a single panmictic humpback population.

i. Feeding Areas

SPLASH results have further informed observations made from previous studies and allowed the recognition of more robust feeding area definitions. Humpback whales show a high degree of feeding site fidelity within a feeding area and relatively low

interchange rates with other areas. The interchange that does occur appears to decrease as a function of geographic distance, meaning that individuals seen in multiple feeding grounds were most often previously seen in the adjacent feeding areas. Any interchange that has been observed between feeding areas is discussed in each regional section below.

California and Oregon

The feeding area boundary between the humpback whales feeding off British Columbia and those feeding off the US coast has been debated. Until recently, most studies described California, Oregon and Washington as one feeding group (Calambokidis *et al.*, 1996; Calambokidis *et al.*, 2000b). Now it appears that the distinction is better supported as one group feeding offshore of California and Oregon and another feeding offshore of northern Washington and southern British Columbia (Calambokidis *et al.*, 2008).

Humpback whales are generally seen off the coast of California and Oregon in spring, summer and fall. Most sightings of humpback whales have been in coastal waters, often within 30nmi of the shoreline (Calambokidis and Barlow, 2004). Areas of particularly high concentration of humpback whales were found around the Farrallon Islands, north and south of San Francisco Bay and around Point Conception (Calambokidis *et al.*, 2004). However, humpback whales were also detected in waters off California (80-100nmi) during winter and early spring aerial surveys (Forney and Barlow, 1998). Photo-identification of some of these individuals has revealed that most of the whales occurring in these waters are part of the California feeding aggregation.

British Columbia and Northern Washington

Recent results from the SPLASH study support the grouping of the feeding area off northern Washington with southern British Columbia feeding areas and their distinction from a northern British Columbia feeding area (Calambokidis *et al.*, 2008). This classification was supported by the presence of only one photographic identification match of a humpback whale across both northern and southern British Columbia (Calambokidis *et al.*, 2008). Gregr *et al.* (2000) suggested that the British Columbia feeding area may be increasing in abundance slowly due to immigration from other neighboring feeding areas (e.g., southeastern Alaska).

An analysis of historical whaling data from British Columbia whaling stations on Vancouver Island and the Queen Charlotte Islands suggests that a resident subpopulation of humpback whales previously existed offshore of British Columbia (Gregr *et al.*, 2000). Differences in the timing of historical depletion of this population compared to both the population to the north in southeastern Alaska and the population to the south offshore of California and Oregon support the hypothesis that this was a distinct subpopulation (Gregr *et al.*, 2000). The population was likely small and was depleted quickly over a few seasons. Approximately 200 individuals were taken from around the Strait of Georgia, likely extirpating the population by the early 1900s (Gregr *et al.*, 2000).

Southeastern Alaska

Recent results from the SPLASH study support the grouping of the northern British Columbia feeding area with Southeastern Alaska. Exchange rates between these two regions were the highest observed between any feeding areas in the North Pacific (Calambokidis *et al.*, 2008). Southeastern Alaska supports a large population of humpback whales (Straley *et al.*, 2009). Humpback whales are distributed through all

major waterways of the southeastern Alaska coastline, and annual concentrations of humpback whales are consistently seen in Icy Straight, Lynn Canal, Stephens Passage, Chatham Straight and Frederick Sound (Dahlheim *et al.*, 2009). Humpback whales have been observed in Glacier Bay during each year surveyed (Dahlheim *et al.*, 2009).

Abundance and distribution of humpback whales in southeastern Alaska were observed to follow a strong seasonal pattern (Straley *et al.*, 2009). Humpback whales increased in number throughout the spring and were found to congregate in particular areas such as those near Icy Straight, Frederick Sound and Stephens Passage (Dahlheim *et al.*, 2009). As the number of humpback whales increased over the summer months, the distribution of whales was found to spread throughout the region more evenly. Numbers remained high through the fall season (Dahlheim *et al.*, 2009). Mean group size varied significantly across years in this region as well as across seasons, with the smallest groups occurring in the spring and the largest in the fall (Dahlheim *et al.*, 2009).

Northern Gulf of Alaska, Aleutian Islands and Bering Sea Feeding Areas

Feeding areas west of southeastern Alaska are understudied compared to those to the east. However, it is known that whaling resulted in loss of large numbers of humpback whales from the Gulf of Alaska, the Alaska Peninsula and the Aleutian Islands (Zerbini *et al.*, 2006b). Feeding areas west of southeastern Alaska known to be occupied by humpback whales today include the northern Gulf of Alaska, the western Gulf of Alaska, the Bering Sea, the Aleutian Islands.

Most humpback whales showed a high degree of site fidelity to these feeding areas. Of the few within-season interchanges between feeding areas that were observed, the northern Gulf of Alaska was found to have some interchange with southeastern

Alaska and the western Gulf of Alaska (Alaska Peninsula area) (Calambokidis *et al.*, 2008). Additionally, the Eastern Aleutians and the southern Bering Sea also had relatively high rates of within-season interchange (Calambokidis *et al.*, 2008). Between seasons, interchange was observed between the northern and western Gulf of Alaska at an intermediate rate (Calambokidis *et al.*, 2008).

Russia Mainland and Commander Islands feeding areas

As part of the SPLASH project, surveys were conducted around the Commander Islands and along the Kamchatka Peninsula north into the Bering Sea. Humpback whales were found in three main regions; the Commander Islands, one area off the east side of Kamchatka and in the Gulf of Anadyr at the northern end of the Bering Sea (Calambokidis *et al.*, 2008).

ii. Breeding Areas

Humpback whale breeding and calving occurs in three broad regions in the North Pacific: the eastern North Pacific, the Hawaiian Islands and the western North Pacific. Some degree of interchange exists within each of these breeding areas (e.g., between Mainland Mexico and Baja California Mexico). However, the degree of interchange within each breeding area varies substantially among regions. Results from the SPLASH study suggest that Hawaii is one breeding region, rather than multiple breeding regions, given the amount of exchange between islands. However, the western and eastern North Pacific breeding areas show a higher degree of structure and isolation of sub-areas within each region.

Movement between these three broad breeding regions also exists, though frequency of exchange is thought to be low. The SPLASH study found that two whales

moved between the western North Pacific breeding region and Hawaii, and that 17 moved between the eastern North Pacific breeding region and Hawaii (Calambokidis *et al.*, 2008). Calambokidis *et al.* (2001) found four transits of three individual whales between Japan and Hawaii as well as six transits of five individual whales between Mexico and Hawaii (three from the Islas Revillagigedos and two from Baja California). Sightings of the same whale on different breeding grounds were always in different years.

Hawaii

A high degree of interchange between waters off each of the principal islands has been observed for humpback whales in Hawaii. This low island-specific fidelity suggests that Hawaii represents a single breeding region. The amount of interchange between islands does not show a simple relationship with geographic distance (Calambokidis *et al.*, 2008).

Western North Pacific

Of the three breeding regions, the western North Pacific breeding region remains the least studied. Historically, humpback whales were caught in the winter around Taiwan, Hainan, the Ogasawara, Mariana, Marshall and Ryukyu Islands (Darling and Mori, 1993b). Recently, humpback whales have also been observed in Okinawa and Ogasawara, at a more northerly location than the other Western North Pacific breeding area. This may be the northern remnant of a larger pre-whaling distribution across the region. Sampling effort at these regions in recent years revealed that Ogasawara and Okinawa were distinct from one another, with a small degree of interchange both within and between years (Calambokidis *et al.*, 2008). Additionally, humpback whales have

been observed in the Philippines, significantly south of these areas. Currently, there are few records of humpback whales offshore off Taiwan and Saipan.

Eastern North Pacific

The breeding region in the eastern North Pacific includes mainland Mexico, the Baja California Peninsula, Mexico, the Revillagigedos Islands, Mexico and Central America. Rates of interchange vary among these four regions. Based on interchange and migratory destination information, the Baja and Mainland Mexico populations had previously been grouped together as a coastal population separate from the Revillagigedos Islands population (Urban *et al.*, 2000; Urban-R and Aguayo L, 1987). Recent results from the SPLASH study indicate that Baja California and the Mainland were not significantly different genetically, nor were Baja California and the Revillagigedos Islands. However, the Revillagigedos Islands and the Mainland were significantly differentiated (at $p<0.05$) (Baker *et al.*, 2008). Baja California may be both a breeding destination for some whales and a migration route for whales destined for other breeding destinations in the eastern North Pacific.

The other eastern North Pacific breeding area for humpback whales exists offshore of Central America along the western coasts of Costa Rica, Panama, Guatemala, El Salvador, Honduras, and Nicaragua (Calambokidis *et al.*, 2008; Rasmussen *et al.*, 2002).

B. Migration

Much research effort has been focused on the population structure of humpback whales in the North Pacific. Strong fidelity to both feeding and breeding sites has been observed, but movements between feeding and breeding areas are complex and varied.

An overall pattern of migration has recently emerged. Asia and Mexico/Central America were found to be the dominant breeding areas for humpback whales that migrate to feeding areas in lower latitudes and more coastal areas on each side of the Pacific, such as California and Russia (Fig. 2.2). The Revillagigedo Archipelago and Hawaiian Islands were the primary winter migratory destination for humpback whales that feed in the more central and higher latitude areas (Calambokidis *et al.*, 2008). However, there were exceptions to this pattern, and it seems that complex population structure and strong site fidelity coexist with lesser known, but potentially high, levels of plasticity in the movements of humpback whales (Calambokidis *et al.*, 2008). Additionally, the SPLASH data suggested that there is a yet undiscovered breeding area in the North Pacific, as humpback whales from the Aleutian Islands and the Bering Sea were not well represented in the samples from any breeding area (Calambokidis *et al.*, 2008).

Individuals from numerous breeding areas are found in the same feeding area. When considered by breeding region, migrations have been documented from Central America to northern Washington-southern British Columbia and California-Oregon; Mexico to every feeding ground; Hawaii to every feeding ground and Japan to every feeding ground except California-Oregon and southeastern Alaska. Many of these connections were based on observations of only a few individuals, and as a result it is not known how common some of these patterns may be. Taking into account the subdivisions within the breeding regions, a higher degree of feeding area specificity is apparent.

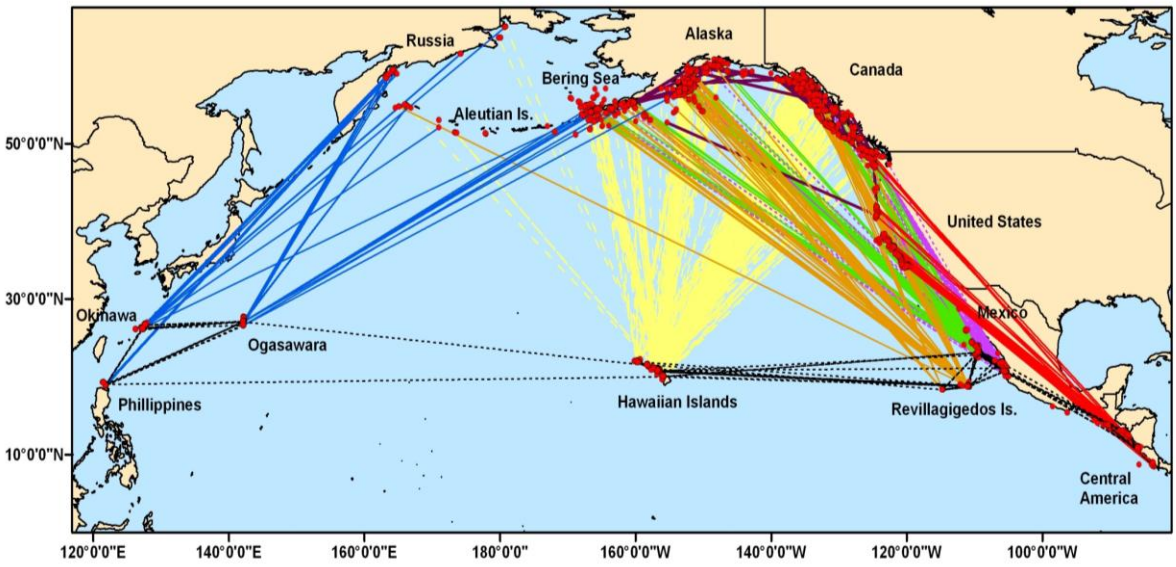

Fig. 2.2: Migratory connections identified by photographic matches between breeding and feeding grounds. Straight lines connecting sighting locations are not meant to illustrate actual migration pathways. Migratory connections are color-coded by breeding ground. (Calambokidis *et al.*, 2008)

C. Genetic Differentiation

A high degree of genetic differentiation exists among most humpback whale feeding area aggregations within the North Pacific basin. Analysis of Molecular Variance (AMOVA) of mtDNA haplotypes showed significant differences among individuals from 8 feeding areas (overall F_{ST} =0.179, $p<0.001$) (Baker *et al.*, 2008) (Table 1). Sample sizes in a few regions were too small for comparison, but where these were adequate, pair-wise F_{ST} comparisons revealed that nearly all feeding aggregations were significantly distinct from one another, with a few exceptions (Baker *et al.*, 2008) (Table 2.1).

Table 2.1: Levels of differentiation between Feeding Areas (bold indicates significance at 0.05) (From Baker *et al.*, 2008)

	Russia	**Bering Sea**	**E. Aleutians**	**W. Gulf Alaska**	**N. Gulf Alaska**	**SE AK**	**N. BC**	**S. BC-Wash**
Bering	**0.094**	--						
E. Aleutians	**0.114**	-0.012	--					
W. Gulf Alaska	**0.039**	0.012	0.010	--				
N. Gulf Alaska	**0.105**	0.013	0.007	**0.014**	--			
Southeast Alaska	**0.389**	**0.242**	**0.343**	**0.220**	**0.116**	--		
N.British Columbia	**0.293**	**0.174**	**0.245**	**0.148**	**0.080**	0.003	--	
S. British Columbia/WA	**0.038**	**0.088**	**0.104**	**0.035**	**0.076**	**0.314**	**0.223**	--
California/ Oregon	**0.268**	**0.157**	**0.108**	**0.202**	**0.229**	**0.478**	**0.401**	**0.268**

Table 2.2: Levels of mitochondrial control region genetic differentiation among breeding grounds and migratory corridors in the North Pacific. From Baker et al., 2008. All values shown are pair-wise FST values for frequencies of control region haplotypes of humpback whale mtDNA. Bold indicates significance at 0.05 while empty fields indicate inadequate sample sizes for this comparison.

	Phil.	**Okinawa**	**Ogasawara**	**HI**	**Mex-Rev.**	**Mex-Baja**	**Mex-Main**	**Cent Am.**
Okinawa		-----						
Ogasawara		**0.032**	-----					
Hawaii		**0.236**	**0.142**	-----				
Mexico-Revillagigedo		**0.128**	**0.046**	**0.043**	-----			
Mexico-Baja		**0.120**	**0.044**	**0.054**	0.003	-----		
Mexico-Mainland		**0.202**	**0.093**	**0.084**	**0.032**	0.005	-----	
Central Am.		**0.454**	**0.328**	**0.282**	**0.223**	**0.148**	**0.068**	-----

Table 2.3: Levels of differentiation between breeding and feeding grounds in the North Pacific (bold indicates significance at 0.05) All values shown are pair-wise F_{ST} values for frequencies of control region haplotypes of humpback whale mtDNA. **Bold** indicates significance at 0.05. Empty fields indicate inadequate sample sizes for this comparison. (From Baker *et al.*, 2008)

	Russia	**Bering**	**E. Aleutians**	**W. Gulf of AK**	**N. Gulf Of AK**	**SE AK**	**N. BC**	**S. BC-Wash**	**Cal-Oregon**
Philippines	--	--	--	--	--	--	--	--	--
Okinawa	**0.031**	**0.200**	**0.283**	**0.130**	**0.198**	**0.577**	**0.497**	**0.127**	**0.360**
Ogasawara	0.002	**0.101**	**0.118**	**0.042**	**0.111**	**0.326**	**0.253**	**0.029**	**0.297**
Hawaii	**0.135**	**0.029**	0.025	**0.033**	0.000	**0.096**	**0.065**	**0.097**	**0.252**
Mex-Rev	**0.042**	0.010	0.008	-0.006	**0.021**	**0.234**	**0.162**	**0.048**	**0.206**
Mex-Baja	**0.042**	0.015	0.002	0.000	**0.032**	**0.246**	**0.176**	**0.045**	**0.152**
Mex-Main	**0.088**	0.018	-0.002	**0.031**	**0.059**	**0.366**	**0.272**	**0.095**	**0.079**
Central Am	**0.302**	**0.168**	**0.109**	**0.218**	**0.250**	**0.625**	**0.527**	**0.303**	-0.014

Breeding ground comparisons, for which there were adequate sample sizes, showed that all areas were genetically distinct from one another with the exception of Baja California, Mexico which did not differ significantly from the Revillagigedos Islands or mainland Mexico regions (Baker *et al.*, 2008) (Table 2.2). Additionally, the sample sizes for Okinawa and the Phillipines were small but the two populations did not differ significantly from each other (Table 2.2).

Comparisons among most breeding and feeding areas also showed significant genetic differences, even for areas with strong migratory connections (Table 2.3). Though some known migratory pathways were supported by the genetic comparisons, (California-Oregon feeding area did not differ significantly from those sampled in the

Central America breeding area), individuals from multiple breeding grounds are found in each feeding ground, causing the significant genetic differences between most breeding and feeding grounds (Table 2.3).

D. Discreteness & Significance Determinations

Based on the above information on distribution, population structure, migratory connections and genetic differentiation, presented in greater detail in Fleming & Jackson, 2011, the BRT concluded that six populations of humpback whales in the North Pacific meet the established criteria for being discrete under the DPS policy guidelines. Discreteness may be established by physical, physiological, ecological or by behavioral differences or separation by international boundaries. The BRT focused on breeding populations as units that could be designated as DPSs since the ESA describes species and DPSs as units that "interbreed when mature". However, information on feeding location and migratory connections was considered in the identification of DPSs. The six discrete units are:

(1) Central America
(2) Mainland Mexico
(3) Revillagigedos Islands
(4) Hawaiian Islands
(5) Okinawa and Philippine Islands pooled
(6) Unidentified breeding area in the western North Pacific

Once it is determined that a population is discrete, the significance of the population must be assessed. Significance may be established if the population persists in a unique setting, if the loss of that segment would cause a gap in the species range, if the population shows genetic differentiation or if it represents the only remaining natural occurrence of the species. One of these four factors must be met in order to establish

significance. Table 2.4 summarizes the significance assessment for each discrete unit. Though Mainland Mexico and the Revillagigedos were both determined to be discrete, neither of them independently met any of the significance criteria and therefore these two population units were combined into a single Mexico unit.

Table 2.4: Significance of Discrete Units by Factor.

[1] The Mexico segment is a combination of mainland Mexico and Revillagigedos Islands units, and taken together is considered a DPS
[2] One dissenter
[3] Uncertainty about location and population size, which in part drove the vote to "yes"
[4] Markedly different in haplotype frequencies from most other segments in the subspecies
[5] The team noted that mainland Mexico segment contains a high level of haplotype diversity

Discrete Unit Name	Factor 1 persists in a unique ecological setting	Factor 2a loss would result in a significant gap in breeding range	Factor 2b loss would result in a significant gap in feeding range	Factor 3 is the only natural occurrence of humpback whales	Factor 4 differs markedly from other populations in genetic characteristics	This discrete population unit is a DPS
Hawaii		X	X			X
Central America	X[2]	X			X[4]	X
Mainland Mexico						
Revillagigedos Islands						
Mexico[1]		X				X
Okinawa/Philippines		X[3]	X			X
Second West Pacific		X[3]				X

Summary of Distinct Population Segments

After both discreteness and significance were assessed, the 5 resulting discrete population segments were Hawaii, Central America, Mexico, Okinawa/Philippines and a 2nd West Pacific DPS. The specific discreteness and significance criteria for each DPS are summarized in Table 5. While there was consensus on the BRT regarding Hawaii, Mexico, and Central America, the Western Pacific breeding grounds were slightly more

complex. Although sample sizes from the Philippines were small, this population did not differ significantly from Okinawa genetically and was therefore pooled with Okinawa. Ogasawara was determined to be a migratory path through which individuals from both the Okinawa/Philippines population and the 2nd West Pacific population may transit. The 2nd West Pacific breeding area is unknown but inferred from the low number of matches of individuals sighted in the Aleutian Islands and the Bering Sea feeding grounds to any breeding ground.

Table 2.5: Summary of each of 5 DPSs that were identified by the BRT including the factors that met the discrete and significant criteria.

DPS	Breeding Distribution	Feeding Grounds	Why is the unit "discrete"?	Why is the unit "significant"?
Hawaii	Hawaiian Archipelago	Primarily SE Alaska; observed in all	- Significant genetic differentiation - Low rates of movements between other breeding grounds and HI	- Loss would result in major gap in range at both breeding grounds (central N. Pacific) and feeding grounds (SE AK)
Central America	Pacific coast of Costa Rica, Panama, Guatemala, El Salvador, Honduras, Nicaragua	Almost exclusively California and Oregon; some observations in Washington/S. British Columbia	- Significant genetic differentiation - >85% match rate between Cent America and CA/OR	- Shares some mtDNA haplotypes with a Southern Hemisphere DPS and may be a conduit for gene flow (ie. "unique ecological setting") - Loss would result in significant gap in breeding range
Mexico	Pacific coast of mainland Mexico, Baja CA, Revillagigedos Islands	Ranges from CA to the Aleutians	-Significant genetic differentiation - Low rates of movement between other breeding grounds and Mexico	- Loss would result in significant gap in breeding range

Table 2.5 cont.: Summary of each of 5 DPSs that were identified by the BRT including the factors that met the discrete and significant criteria.

DPS	Breeding Distribution	Feeding Grounds	Why is the unit "discrete"?	Why is the unit "significant"?
Okinawa/ Philippines	Okinawa/Phillipines;	Primarily Russian coast, some observations in the Bering Sea and Aleutian Islands	- Significant genetic differentiation - Low rates of exchange with other breeding grounds	- Loss would result in significant gap in breeding range and feeding range
2nd West Pacific DPS	Unknown	Aleutian Islands	- Apparent low exchange with other breeding grounds	- Loss would likely result in large gap in breeding and feeding range

III. Extinction Risk Assessment

A. Abundance

The most current estimate of abundance for the entire North Pacific basin, resulting from the SPLASH project, is 21,063 individuals (CV=0.04) (Barlow *et al.*, 2011; Calambokidis *et al.*, 2008) This is significantly larger than any previous estimates for the basin and is greater than some of the published estimates of pre-whaling abundances (Rice, 1978). This estimate has been corrected for some known biases, and although other biases may be influencing this estimate, they are likely to be negative, making this estimate a conservative one (Barlow *et al.*, 2011). Regional estimates of abundance are presented in Table 2.6.

Table 2.6: Estimated levels of abundance for each DPS are shaded**.** If there is uncertainty in the abundance level of the DPS, all categories that could apply are shaded. The number of mature individuals was estimated as one-half of the total population size.

Population level	**>1000 mature individuals (>2000 total)**	**< 1000 mature individuals (<2000 total)**	**<250 mature individuals (<500 total)**	**<50 mature individuals (<100 total)**
Pacific Ocean				
Hawaii				
Central America				
Mexico				
Okinawa/ Philippines				
Second West Pacific				

B. Trends

Trends in abundance have been calculated for some regions of the North Pacific as well as for the North Pacific overall (Table 2.7). Besides the SPLASH study, the only other mark-recapture effort to examine North Pacific abundance on a basin scale was the NPAC study based on photographic identifications of individual whales from 1990-1993 from 3 wintering regions (Hawaii, Mexico, Japan) and feeding areas from California to the Aleutian Islands (Calambokidis *et al.*, 1997). Comparing the NPAC best estimate of 6,010 to the SPLASH results gives an estimate of 4.9% annual increase over the 13-year time span. If the SPLASH results are compared to the basin-wide estimate made in 1966 by Johnson and Wolman (1984) of approximately 1,200 individuals, a 6.8% annual increase is found for the 39-year time span (Calambokidis *et al.*, 2008).

Other growth rates have been calculated on more regional scales including ~8% per year for the U.S. West Coast from 1991-2008 and 6.6% per year for the Alaskan Peninsula and Aleutian Islands from 2001-2003 (Calambokidis, 2009; Zerbini *et al.*,

2006b). Between 1991 and 2007, a 10.6% annual increase in population size was calculated for southeastern Alaska (Dahlheim *et al.*, 2009).

Using regional estimates from the NPAC study (1990-1993) and the SPLASH study (2004-2006), trends were calculated for Hawaii and Asia. The humpback whale population found in waters off Hawaii showed an annual growth rate of 5.5-6.0%, and an annual growth rate of 6.7% was observed in the western Pacific population (Calambokidis *et al.*, 2008). The western Pacific estimate is less robust, however, as sampling effort was significantly greater in the SPLASH study, which may bias the western Pacific estimate upwards (Calambokidis *et al.*, 2008).

Table 2.7 Summary of trends in abundance for each DPS. The category of trend that the DPS is thought to be undergoing is shaded. If there is substantial uncertainty in the trend, all categories that may apply are shaded. If no reliable information on trend for the DPS is available, the last column ("unknown") is shaded.

Population trend	Increasing Strongly	Increasing moderately	Stable/little trend	Declining	Unknown
Pacific Ocean					
Hawaii					
Central America					
Mexico					
Okinawa/ Philippines					
Second West Pacific					

C. Threats and anthropogenic impacts

Specific information on threats is not available for all areas and habitats in the North Pacific occupied by humpback whales throughout their life cycle (Table 2.8). Significantly more data, observations, and reporting are available from US waters in relation to human-related threats than from other regions in the North Pacific. Though the

information is low in some areas, it is clear that threats are present. For example, SPLASH photographs found over 20% of individuals showed signs of entanglement scarring in all known feeding areas in the North Pacific with some areas having greater than 50% scarring rates. The paucity of information on threats and their corresponding magnitudes on the high seas and in the waters of other nations should be considered when examining population abundances, structure and trends.

Table 2.8: Summary of all threat levels facing each DPS color coded by the severity of the threat, as determined by the BRT.

Severity of threat
4= Threat is likely to eliminate the DPS
3 =High, threat is likely to seriously reduce the population size or the growth rate of the population.
2= Medium, threat is likely to moderately reduce the population size or the growth rate of the population.
1=Low or none, threat is likely to have no or minor impact on population size or the growth rate.
unkn = Severity of threat is unknown
* = trend of threat is increasing

		coastal development	Contaminants	energy exploration &	competition with fisheries	offshore aquaculture	Whaling	whale watching	Scientific research activities	Killer whale and shark predation	Disease	Parasites	Anthropogenic Noise	Vessel collisions	Fishing gear entanglements	Climate Change	HABs
N. Pac.	Hawaii	1*	1	1*	1	1*	1	1	1	1	1	1	1*	1*	2	unkn*	1*
	Central America	1*	1	1	1	1	1	1	1	1	1	1	1*	2*	2	unkn*	1*
	Mexico	1*	1	1	1	1	1	1	1	1	unkn	unkn	1*	1*	2*	unkn*	1*
	Okinawa/Phil.	1	1	2*	2	unkn	2*	1	1	1	unkn	unkn	1*	2*	3	unkn*	unkn
	2nd W. Pac	1	unkn	unkn	unkn	unkn	unkn	1	1	unkn	unkn	unkn	1*	unkn	unkn	unkn*	unkn

D. Recovery from Exploitation

Most humpback whale populations in the North Pacific were depleted significantly during the first half of the 20th century due to whaling from shore stations and factory ships (Clapham *et al.*, 1997; Gregr, 2000; Witteveen *et al.*, 2004; (Darling and Mori, 1993b). Some populations were targeted a second time in the early 1960s before substantial recovery had occurred, further reducing population sizes. An estimated 28,000 humpback whales were removed from the North Pacific in the 20th century before the species was placed under international protection (Rice, 1978). Remaining population sizes may have been as low as 1,000 to 1,400 humpbacks (Gambell, 1976; Johnson and Wolman, 1984). The number of individuals removed is likely an underestimate because of under-reporting by Soviet whaling (Yablokov, 1994). Russian whaling continued in the North Pacific until 1980 (Zemsky *et al.*, 1995).

Two populations that have calculated trends in the North Pacific, seem to be increasing. Though there is no comprehensive assessment of the impact of whaling and the number of individuals removed, it appears clear that in most regional feeding and breeding areas, numbers remain lower than pre-exploitation abundances. Additionally, some geographic areas where humpback whales used to be observed do not appear to have been re-colonized (Gregr *et al.*, 2000).

E. Risk Assessment Determinations

For each DPS, risk of extinction was evaluated by the BRT over a time frame of 3 generations (~60+ years) by considering data on threats, abundance and trends. Three risk categories were defined and the BRT used a structured decision-making approach to assess each DPS. Each member of the BRT was given 100 points to distribute among the

risk categories according to their certainty level regarding the extinction risk of each DPS. Each BRT member arrived at their own determinations of extinction risk, all of these individual votes were then averaged for the final determination (Table 2.9). For DPSs with more evenly distributed points across risk categories there is less certainty regarding DPS extinction risk. The three risk category definitions are:

High Risk: a species or DPS's productivity, spatial structure, genetic diversity, and/or a level of abundance place(s) its persistence in question. The demographics of a species/DPS at such a high level or risk may be highly uncertain and strongly influenced by stochastic and/or small population effects. Similarly, a species/DPS may be at high risk of extinction if it faces clear and present threats (*e.g.*, imminent destruction, modification, or curtailment of its habitat; or disease epidemic) that are likely to create an imminent risk of extinction.

Moderate Risk: a species or DPS is at moderate risk of extinction if it exhibits characteristics indicating that it is likely to be at a high risk of extinction in the future. A species/DPS may be at moderate risk of extinction due to projected threats and/or declining trends in abundance, productivity, spatial structure or diversity.

Not at Risk: a species or DPS is not at risk of extinction.

Table 2.9: Risk assessment results from BRT structured voting.

Distinct Population Segment	High Risk	Moderate Risk	Not at Risk
Hawaii	0%	2%	98%
Central America	28%	56%	16%
Mexico	0%	8%	92%
Okinawa/Philippines	36%	44%	21%
Second West Pacific	14%	47%	39%

It was concluded that Hawaii and Mexico are not at risk of extinction, largely due to the magnitude of threats in relation to their large population size and known positive growth rates. The Central America DPS has a small population size and an unknown growth rate. In addition, historical whaling records suggest that this population was heavily targeted by whaling operations and remains well below pre-exploitation abundances today. The BRT had less certainty about extinction risk for this population since growth rate is unknown and population size is small but the majority of votes was for moderate risk. The Okinawa/Philippines DPS is between moderate and high risk of extinction since threats are numerous and expected to increase and population sizes and ranges remain reduced from pre-exploitation levels. Lastly, the 2nd West Pacific population is likely to be at moderate to low risk of extinction. The high level of unknowns surrounding this population resulted in greater uncertainty regarding this conclusion.

Presently, the point distributions shown in Table 2.9 reflect a qualitative assessment made by a panel of experts and these are used by NMFS policy-makers to decide whether a DPS should be listed as endangered, threatened or not warranted of listing under the ESA. While these extinction risk assessments seem rather subjective, there is significant research effort underway to create more robust quantitative metrics for determining population extinction risks (Taylor, 1997; Taylor *et al.,* 1997; Waples *et al.,* 2007). This should help standardize future ESA listing determinations.

IV. Reflections on the Science-Policy Interface

Undoubtedly, the greatest challenge in making resource management policy decisions is dealing with uncertainty. Uncertainty may surround many aspects of

humpback population biology – the boundaries of a population's range, abundance and growth rates. Or uncertainty may be most pronounced in the degree of human impact on a population's longevity. This uncertainty results simply from a paucity of data. However, it remains unrealistic to assume that most cetacean populations will ever be well-studied enough to fill all the data gaps. Therefore, management will always be conducted in the face of uncertainty.

In order to minimize this uncertainty, basic research on population structure, abundance and trends is critical. Though it often lacks broad funding appeal, it is likely to become increasingly important as direct and indirect human impacts on ocean ecosystems increase in magnitude over the coming decades, necessitating escalated and adaptive management actions. Management decisions depend on long-term continuous datasets. Funding cuts that result in missing years of data may have drastically reduce detection of trends in abundance and movements of populations. Information on population structure is always the first line of data required for population assessment and therefore may be a valuable and strategic research area to prioritize. For cetaceans, investigations of population structure require extensive time at sea and numerous re-sampling events of individuals in order to decipher population range, connectivity, and migratory behavior. Developments of new tools that detect demographically discrete populations and may require less repeat sampling could be beneficial to management interpretations. Ecological markers of an individual's location such as stable isotopes or contaminants can provide additional data on population structure at shorter temporal and finer spatial scales than genetic data and are useful additional lines of evidence. Resolving links between ecosystem conditions and cetacean distribution may improve predictions of

population movement under future oceanographic conditions and inform corresponding interpretations of abundance and trends. Though more research is needed to test the ability of these various tools to detect population units relevant to management, they are promising contributions and may help reduce uncertainty in population assessments.

Once population determinations have been made at the scientific level, these are passed to policy-makers. At that stage, the identification of populations and the resulting management actions may differ from the scientific recommendations. Populations may often be "re-combined" due to logistical, economic or political considerations. While these decisions ultimately lie outside of the scientific arena in many ways, policy decisions are guided by the scientific determinations which in turn are only as certain the available data allow them to be. Therefore, it should remain a priority for wildlife biologists to structure scientific data collection efforts to be well-poised for answering basic fundamental questions essential to population biology and management.

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Chapter 3.

Interannual variability in humpback whale stable isotope ratios reflecting diet in response to ocean-climate: implications for discerning population structure

Abstract

Conservation of marine species requires an understanding of population structure and identification of appropriate management units. Discrete populations of humpback whales have previously been identified through spatial variability in isotopic signatures; however, these isotopic studies of population structure have largely been conducted over one to two years. The long-term consistency of a population's isotopic signatures has not been examined. Therefore, the reliability of stable isotopes as population markers remains uncertain despite their increasing use in studies of population structure. In this study, we examined carbon and nitrogen stable isotope signatures in the skin of humpback whales over an eighteen-year period (1993-2010) using 174 skin biopsies collected from a single feeding population off central California. Humpback whales showed significant inter-annual variability in isotope signatures across the study period ($\delta^{15}N$: $F_{13,157}=10.29$, $p<0.0001$; $\delta^{13}C$: $F_{13,156}=18.55$, $p<0.0001$). We tested the population assignment of our California samples using a classification model that was previously constructed based on 2004 and 2005 data to identify six isotopically distinct North Pacific feeding groups. The high degree of temporal isotopic variability in our California samples significantly impacted population structure interpretations ($\chi^2_{13}=46.05$, $p<0.0001$). The 2004-2005 classification tree correctly predicted the feeding location of only 33% of 1993-2010 California samples. Excluding our 2004-2005 samples, our correct classification declined to 8%. The temporal variability observed appears to be

driven largely by oceanographic changes in the CCE. 2001-2003 humpback biopsy samples reflected measured carbon and nitrogen values of krill, and were significantly different from 2004-2006 samples which reflected measured isotopic values of schooling fish. This shift coincides with a cool-to-warm phase-shift in the Pacific Decadal Oscillation and an accompanying change in the dominance of prey from krill to schooling fish, providing further evidence that top predators may be useful indicators of oceanographic conditions. Since many studies have relied upon isotope data from past analyses to interpret current geographic assignments, trophic structure or create isoscapes, this work provides an important cautionary tale that temporal variability should be considered before applying stable isotope analysis to marine mammal science.

Introduction

Thorough understanding of species population structure is necessary for any study of population dynamics. Since population structure is governed by both evolutionary and ecological processes, resolution of population structure is improved with multiple lines of evidence, including genetic and ecological data (Geffen *et al.*, 2004; Howeth *et al.*, 2008). Cetaceans are highly mobile, adapting their geographic range temporally over seasonal, interannual and interdecadal scales. Additionally, they have a diverse suite of habitat types, ranges and life histories. These factors create complex distribution patterns and population structures. Detection of this population structure is further complicated by the difficulty of accessing and assessing cetacean populations (Redfern *et al.*, 2006). Cetaceans are often in pelagic environments far from shore making sampling difficult and the majority of research programs have fixed survey regions which, in different survey years, may contain variable portions of a population's range (Forney, 2000).

Information on population structure and connectivity is also critical in establishing management units to effect conservation. Reliable information on cetacean population structure is mandated by the US Marine Mammal Protection Act (MMPA) which requires that species and "population stocks" (heretofore referred to as "population") be maintained as functioning parts of their ecosystem. In order to meet this objective, marine mammals are often managed below the species level. Since populations may be demographically though not genetically distinct, other lines of evidence in addition to genetic data are considered in order to identify populations (Taylor, Dizon, 1999).

Stable isotope analysis has recently been applied to studies of cetacean population structure in order to resolve ecological and trophic differences between populations (Hobson, 2006; Rocque *et al.*, 2006; Witteveen *et al.*, 2009b). Geographic variability in isotope values create signatures that act as chemical tracers of individual or population movements and foraging history (Farquhar *et al.*, 1989; Newsome *et al.*, 2007; Post, 2002). Carbon stable isotope patterns are primarily caused by processes associated with photosynthesis and therefore reflect changes in primary production. Near-shore environments tend to be more enriched in $\delta^{13}C$ compared to pelagic habitats allowing carbon stable isotopes to be a good indicator of location (Farquhar *et al.*, 1989; Newsome *et al.*, 2007; Post, 2002). Ratios of $^{15}N/^{14}N$ provide an indicator of relative trophic position. $\delta^{15}N$ becomes more enriched with increasing trophic level because the lighter isotope, ^{14}N, is preferentially excreted during metabolic processes (Farquhar *et al.*, 1989; Newsome *et al.*, 2007; Post, 2002).

These differences in isotope signatures provide resolution of short temporal scale (decadal) and ecosystem-level spatial scale structuring. However, population structure studies employing stable isotope analysis have largely assumed temporal consistency of isotopic signatures in a region while searching for geographic differentiation. This assumption is particularly tenuous in marine environments that are highly dynamic and subject to both episodic and prolonged shifts in local ecosystem conditions that may impact isotopic signatures at all trophic levels (Kurle *et al.,* 2001; Kurle *et al.,* 2011). While significant research effort has already applied stable isotope methods to population structure studies, tests of multiyear and decadal stability of these signatures remain needed for proper interpretation of isotopic differentiation between populations.

Humpback whales (*Megaptera novaeangliae)* provide a good case study for testing long-term consistency of cetacean stable isotope signatures. Humpback whales have complex population structure and perform some of the longest migrations of all mammals, transiting from low-latitude breeding areas occupied in winter to high latitude feeding areas inhabited in the summer months (Baker *et al*., 1993; Baker *et al.,* 1986). Humpback whale population structure has been especially well-studied in the North Pacific through various methods including mark-recapture, genetic and stable isotope signatures (Baker *et al.*, 2008; Calambokidis *et al.*, 2008; Witteveen *et al.*, 2009b). Each feeding area in the North Pacific is ecologically and geographically distinct; most of these areas are upwelling regions characterized by high temporal and spatial variability in physical processes.

Previous work by Witteveen et al. (2009a) demonstrated that most of these feeding-ground destinations of humpback whales in the North Pacific could be

differentiated from one another through stable isotope analysis of humpback whale skin biopsy samples collected over two years (2004-2005). However, humpback whales are known to be largely opportunistic foragers, feeding on euphausiid crustaceans and a wide variety of small schooling fish (Baker *et al*., 1985; Clapham *et al*., 1997; Geraci *et al*., 1989). To build the high-energy reserves needed for fasting during migration and the breeding season, humpback whales require high-density prey patches (Hazen et al., 2009). Their prey selection likely depends on the availability of different prey species. Prey availability varies spatially within and between ecosystems and temporally due to changing oceanographic conditions, with consequences for humpback whale diet. Since different prey species are expected to have different stable isotope compositions, variations in humpback whale diet will likely cause temporal variability in stable isotope signatures of humpback whale populations (Becker *et al.*, 2007; Miller *et al.*, 2013).

Humpback whales that inhabit coastal waters off California and Oregon in the spring, summer and fall are part of a feeding population that has been confirmed by photographic identification to be separate from neighboring populations (Calambokidis *et al.*, 2008). Recent observations of humpback whales in the California Current System (CCS) suggest that this population may switch between a forage fish-based diet and a euphausiid-based diet on annual time scales (Calambokidis, J. pers. comm.). If the diet of this population changes over time, the resulting isotope signatures can be expected to vary as well. If the variability is significant, the ability to use static isotope signatures as a marker of population structure will be compromised. To determine the reliability of stable isotopes as indicators of population differentiation, I measured the stable isotope ratios of humpback whale skin samples in a single feeding population over two decades.

Specifically, I 1) determine the degree of isotopic variability in the diet through analysis of skin tissue samples over time, 2) evaluate whether this variability influences isotope-based population structure interpretations and 3) explore the potential oceanographic and ecological causes of temporal changes in humpback whale isotopic composition and diet.

Methods

Whale Tissue Collection

Humpback whale skin samples were collected from individual whales in the California Current ecosystem between 34° and 42°N latitude and 119° and 125°W longitude from 1993-2012. Sampling only occurred from April to November when humpback whales are known to use this region for foraging. All samples were collected during NOAA Southwest Fisheries Science Center marine mammal survey cruises or by Cascadia Research Collective from small boat platforms. Most samples were collected by biopsy, but a few samples of sloughed skin were obtained opportunistically with a dip net. All biopsy samples were acquired using a modified rifle or crossbow fitted with a hollow-tipped dart and included skin and a thin layer of blubber. For each sample, a unique sample number was assigned and the date, location and other observational data were recorded. Biopsy samples were frozen at -80°C or stored in ethanol (100%) or DMSO upon collection.

Environmental Data

Physical oceanographic indices and prey time series were obtained from the following sources. Monthly sea surface height anomaly data from the California Current Ecosystem Long-Term Ecological Research program were used as a proxy for El Niño Southern Oscillation as it has been found to more accurately represent ENSO dynamics

in the CCS than the Multivariate ENSO index http://oceaninformatics.ucsd.edu/datazoo/data/ccelter/datasets?action=view&id=153. The monthly mean values for the Pacific Decadal Oscillation were obtained from http://jisao.washington.edu/pdo/PDO.latest. Positive values of the Pacific Decadal Oscillation correspond to warmer temperatures in the California Current and reduced coastal biological productivity. The monthly mean values for the North Pacific Gyre Oscillation were obtained from http://www.o3d.org/npgo/. Prey abundance anomaly data were gathered during Central California coast midwater trawl surveys operated by the Fisheries Ecology Division of the Southwest Fisheries Science Center. Data used in this study are the standardized annual anomalies from the log of mean catch rates. Average daily sea surface temperature data were acquired from NOAA's National Data Buoy Center, buoy # 46026 located on the shelf, at 53m water depth, 18nautical miles west of San Francisco. Daily cumulative upwelling index values were obtained from the Pacific Fisheries Environmental Laboratory http://www.pfeg.noaa.gov/products/pfel/modeled/indices/upwelling/NA/data_download.html. For this analysis, the upwelling data collected from 39°N 125°W were used. For each oceanographic variable, an annual anomaly was calculated in order to examine the inter-annual variability while minimizing the potential compounding effect of seasonal variability. Two different anomaly values were explored in the analyses. The first used all months of the year to calculate the annual value while the second used just the summer and fall months when humpback whales feed off California (April to November).

Prey abundance data were collected by the National Marine Fisheries Service annual spring (May-June) rockfish surveys from 1993- present. Approximately 100

midwater trawls are done annually with a geographic focus on the area from south of Monterey Bay to north of Point Reyes, CA. While numerous species were collected, I focus here on krill (*Euphausia pacifica*), anchovy (*Engraulis mordax*) and sardine (*Sardinops sagax*) because they are known humpback whale prey species (Clapham *et al.*, 1997).

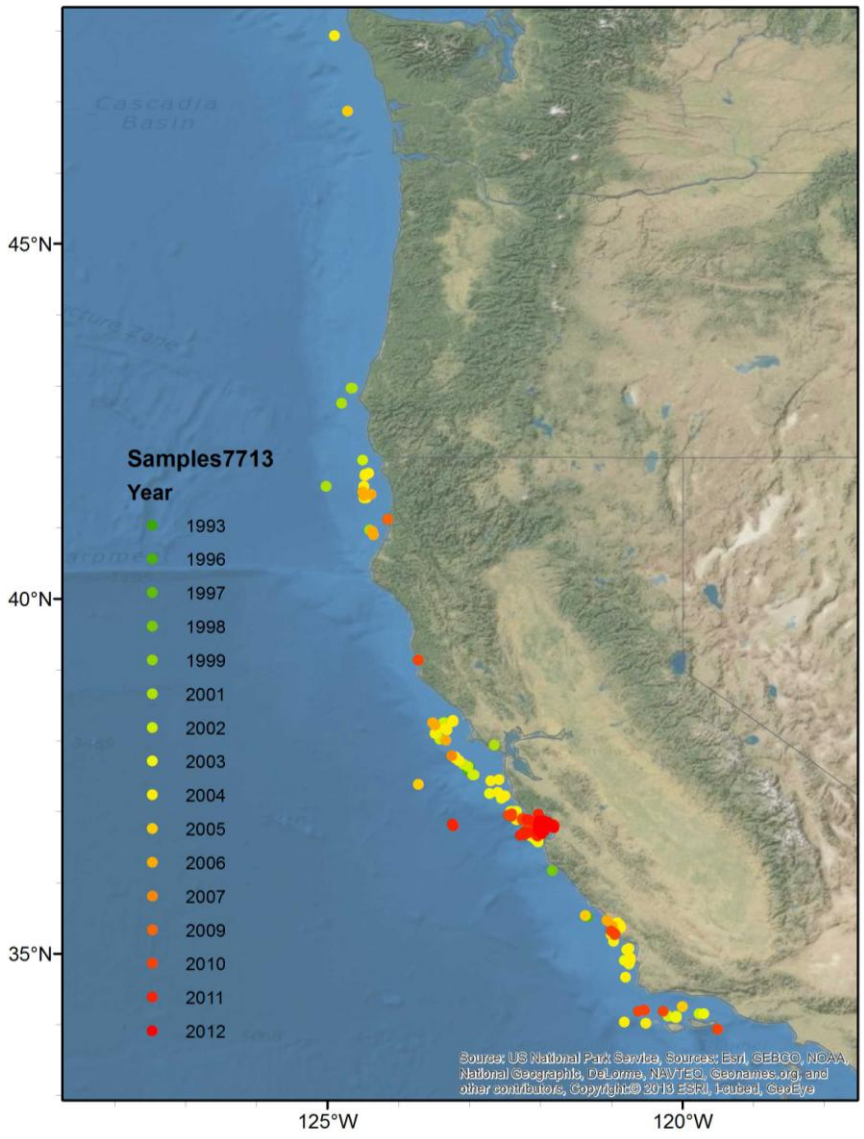

Fig. 3.1: Map of study area with humpback whale sample locations shown color-coded by year of collection.

Isotope Sample Preparation & Analysis

A total of 297 skin samples were analyzed for carbon and nitrogen stable isotope ratios in this study. Approximately 10mg wet weight mass of skin from each biopsy sample was sliced into small pieces and dried for 24 hours in a VirTis benchtop liophilizer. Lipids were extracted using petroleum ether in a Dionex Accelerated Solvent Extractor and proteins were retained for analysis. Approximately 0.4 to 1.0mg of each sample was sealed in tin capsules.

Samples were then analyzed for $\delta^{15}N$ or $\delta^{13}C$ at the University of Florida, Gainesville Stable Isotope Geochemistry Lab. Samples were analyzed by combustion in a Carlo Erba NA 1500 CNS Elemental Analyzer. After combustion in a quartz column at 1000 °C in an oxygen-rich atmosphere, the sample gas was transported in a He carrier stream and passed through a hot reduction column (650 °C) consisting of elemental copper to remove oxygen. The effluent stream from the elemental analyzer then passed through a chemical (magnesium perchlorate) trap to remove water. It was then passed to a ConFlo II interface coupled with a Finnigan-MAT 252 isotope ratio mass spectrometer running in continuous flow mode where the sample gas was measured relative to a laboratory reference gas. Reference materials were Vienna Pee Dee Belemnite and atmospheric nitrogen gas for carbon and nitrogen stable isotope analyses, respectively. USGS40 L-glutamic acid was used as an internal laboratory standard and was run at regular intervals during the analysis to calibrate the system. Stable isotope ratios were then reported as per mil using delta notation determined from the equation $\delta X = [(R_{sample}/R_{standard}) - 1] \times 1000$ where X is ^{15}N or ^{13}C and R is the corresponding ratio

of $^{15}N/^{14}N$ or $^{13}C/^{12}C$ in the sample and standard. The precision of these repeat standard measurements was 0.1‰ for $\delta^{15}N$ and 0.05‰ for $\delta^{13}C$.

Statistical Analysis:

Data were tested for normality using Lilliefor's adaptation of Kolmogorov-Smirnov test for large datasets. Data were tested for homoegeneity of variance using Levene's Test. Results from a subset of individuals of known sex (from the same years) confirmed that sexes did not significantly vary with respect to either $\delta^{15}N$ ($t_{34} = -0.93$, $p=0.36$) or $\delta^{13}C$ ($t_{34} = -0.30$, $p=0.76$). Sex was therefore not considered in subsequent analyses. All statistical tests were performed in R (R package version 2.15.2) and results were interpreted with a significance level of $\alpha = 0.05$.

Inter-annual Variability & Spatial Structure within the CCE humpback whale population

To test for significant differences in isotopic signatures among years, analysis of variance (ANOVA) was used with Tukey's post-hoc test. Although all individual whales sampled in this study are believed to be from the California Current population, the possibility of geographic structure within the CCE population was also explored. The effect of latitude and longitude on both $\delta^{15}N$ and $\delta^{13}C$ was explored using ANOVA. Since there may be interactions between latitude or longitude and some of the other variables considered in this analysis, latitude and longitude were also included in generalized additive models (discussed below).

Implications of Inter-annual Variation on Population Structure Interpretations

In order to test whether interannual variability affected population structure assignment, I built upon previous work by Witteveen et al. (2009a). That study used classification tree analysis to identify isotopically distinct foraging locations of North

Pacific humpback whales based on two years of isotopic sampling, 2004 and 2005. That model incorporated both $\delta^{15}N$ and $\delta^{13}C$ as variables in order to predict feeding group membership for 6 different feeding regions across the North Pacific. I applied that same model to the samples in this study, collected over 19 years from the California Current feeding group, to test whether temporal variability influenced population assignment success. Since all samples used in the present study were collected from the California Current feeding group, any samples assigned, based on isotope signature, to a non-California Current feeding group, would have incorrect population assignment, thereby confirming that temporal variability impacts population assignment.

Environmental causes of humpback whale diet variability

The potential oceanographic and ecological causes of changes in humpback whale diet were explored through a variety of analyses. First, the relationships between carbon and nitrogen stable isotope signatures and oceanographic variables were explored through linear regression analysis. Since changes in climate and oceanographic indices may temporally precede biological responses from top predators, a lagged correlation analysis was also conducted to examine possible time-delayed relationships between humpback whale isotope signatures and ecosystem conditions. Finally, since humpback whale diets are unlikely to respond linearly to changes in habitat, generalized additive models (GAMs) were used to relate the value of isotope signatures to the following habitat variables: sea surface temperature, upwelling index, Pacific Decadal Oscillation index, North Pacific Gyre Oscillation index, sea-surface height anomaly (as a proxy for El Nino Southern Oscillation index), and abundance anomalies of sardine, anchovy and krill. GAMs are nonparametric models that can accommodate many different types of

relationships between the examined variables and are therefore particularly effective at modeling complex ecological relationships. A GAM may be represented as

$$g(\mu) = \alpha + \sum_{j=1}^{p} f_j(X_j)$$

(Hastie and Tibshirani 1990).

where $g(\mu)$ is the link function, and it relates the mean of the response variable given the predictor variables $\mu = E(Y|X1,\ldots,Xp)$ to the additive predictor $\alpha + \Sigma jfj(Xj)$.

A stepwise forward-backward modeling approach was used in the mgcv package within R. Models were compared using Akaike's Information Criterion.

Results

During sample analysis, an error occurred with regard to nitrogen for 1 sample and with regard to carbon for 2 samples. These were removed and therefore, 296 nitrogen and 295 carbon stable isotope samples were included in all statistical analyses (Fig. 3.2).

Year to year variations in sample size reflect differences in sampling effort (Table 3.1). Since the focus of this study was on inter-annual timescales, years with very small sample sizes were left out of most analyses since they may have been insufficient to be representative of an annual signal. The data for both $\delta^{13}C$ and $\delta^{15}N$ did not deviate significantly from a normal distribution ($\delta^{15}N$: D = 0.0468, p-value= 0.1184; $\delta^{13}C$: D=0.0285, p-value=0.811). However, variance across years was not homogenous, indicating differences between years with regards to diet breadth ($\delta^{15}N$: F(1;15)= 3.7164, 5.695e-06; $\delta^{13}C$: F(1;15) = 2.4415, 0.002303).

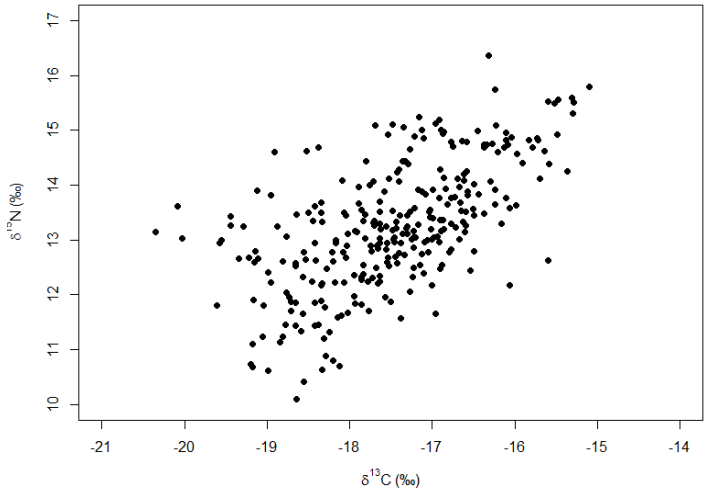

Fig. 3.2: $\delta^{15}N$ and $\delta^{13}C$ for all humpback whale skin samples.

Inter-annual & geographic variability within the CCE humpback whale population

There was no indication of significant geographic variability in $\delta^{15}N$ and $\delta^{13}C$ within the CCE population with regards to latitude (ANOVA: $\delta^{15}N$ $F_{1,289}$=0.443, p=0.506; $\delta^{13}C$: $F_{1,288}$=0.789, p=0.375) or longitude (ANOVA: $\delta^{15}N$ $F_{1,289}$=0.492, p=0.484; $\delta^{13}C$: $F_{1,288}$=0.426, p=0.514). Additionally, latitude and longitude were not included terms in any of the best GAM models for either $\delta^{15}N$ or $\delta^{13}C$. It should be noted, however, that while these results suggest little geographic pattern in $\delta^{15}N$ and $\delta^{13}C$, there may be some degree of geographic variability in humpback whale $\delta^{15}N$ and $\delta^{13}C$ within the sampled individuals but this may have been better explained by the other variables included in the GAM models. For example, prey types may vary by geographic location and therefore relative prey abundance may have better explained the observed whale isotope signatures. This is considered further in the discussion section.

Both nitrogen and carbon varied temporally throughout the study period (Figs. 3.3 and 3.4). Annual means for $\delta^{15}N$ ranged from a minimum of 12.41 in 2012 to a

maximum of 15.12 in 2005 and $\delta^{13}C$ ranged from a minimum in 2001 of -18.76 to a maximum in 2006 of -16.29 (Table 3.1). Humpback whale skin isotope signatures displayed significant differences between sampling years (ANOVA: $\delta^{15}N$: $F_{13,157}=10.29$, $p<0.0001$; $\delta^{13}C$: $F_{13,156}=18.55$, $p<0.0001$). Tukey's post-hoc tests revealed that 24 of the year-to-year comparisons for $\delta^{13}C$ were significant and 20 of the comparisons for $\delta^{15}N$ were significant (Tables 3.2 and 3.3).

Both $\delta^{15}N$ and $\delta^{13}C$ mean values varied to a comparable degree (approximately 2-3 ppm) across the study period and with a similar periodicity (Figs. 3.3. and 3.4). There appear to be two major shifts in isotope signatures. Humpback whale signatures were less enriched during the early part of the study period until 2002 or 2003 when there was a shift in both ratios to more positive values followed by a subsequent drop back to more depleted values from 2010 to 2012, similar to those levels observed in 1993-2002 (Fig. 3.4).

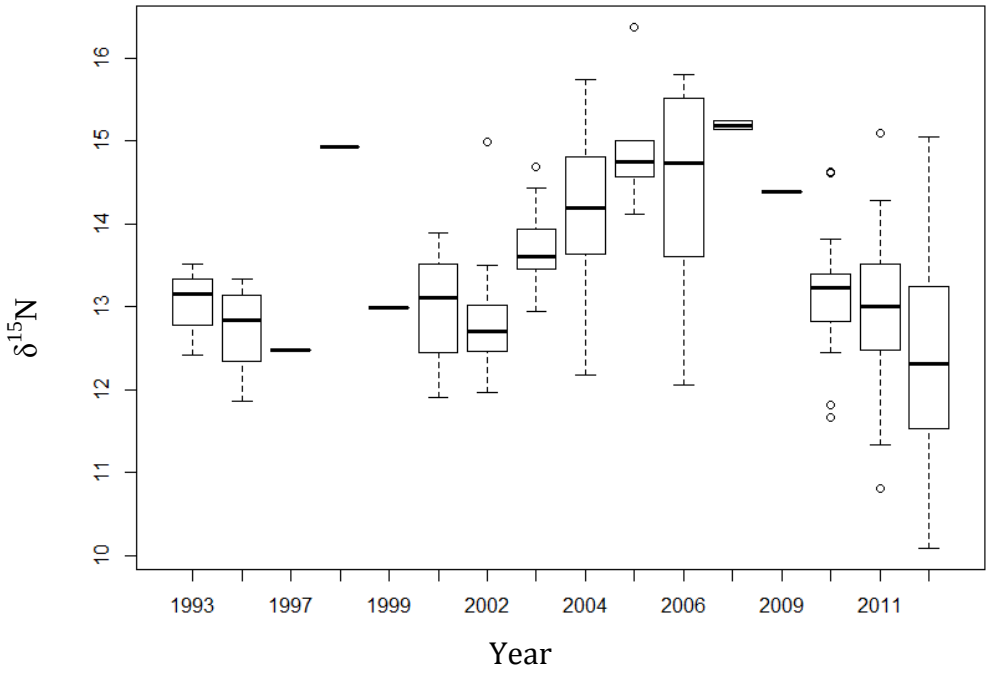

Fig.3.3: $\delta^{15}N$ values measured in 296 skin samples from humpback whales collected in the California Current from 1993-2012.

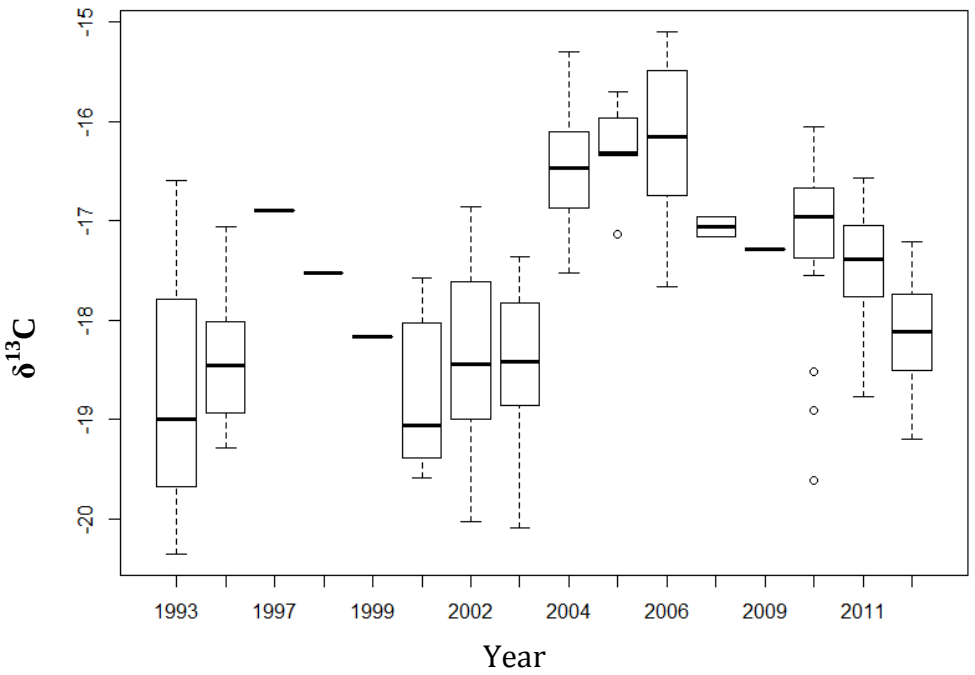

Fig 3.4: $\delta^{13}C$ values measured in 295 skin samples from humpback whales collected in the California Current from 1993-2012.

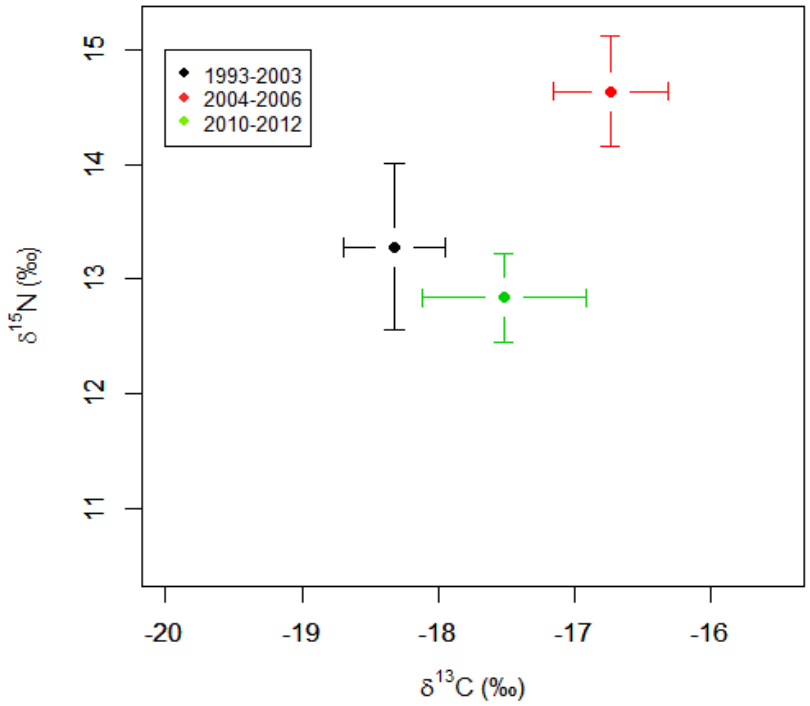

Fig. 3.5: $\delta^{13}C$ and $\delta^{15}N$ values measured in humpback whale skin samples grouped by time period.

Table 3.1. Average values for $\delta^{13}C$ and $\delta^{15}N$ and sample sizes for each year.

Year	N	C	n	SE
1993	13.03	-18.64	3	0.57
1996	12.73	-18.39	8	0.54
2001	12.99	-18.76	8	0.70
2002	12.81	-18.32	20	0.63
2003	13.68	-18.44	15	0.47
2004	14.14	-16.45	59	0.83
2005	15.12	-16.61	9	0.85
2006	14.35	-16.29	17	1.25
2010	13.15	-16.96	23	0.68
2011	12.95	-17.45	65	0.80
2012	12.41	-18.15	67	1.18

Table 3.2. Correlation matrix of pair-wise comparisons resulting from ANOVA and Tukey's post-hoc test for $\delta^{15}N$. Significant values ($p \leq 0.05$) are in bold. Values are symmetrical around the main diagonal, so only one set of values is shown.

Year	1996	2001	2002	2003	2004	2005	2006	2010	2011	2012
1996	NA									
2001	0.999	NA								
2002	1	0.999	NA							
2003	0.322	0.765	0.135	NA						
2004	**0.001**	**0.028**	**<0.001**	0.764	NA					
2005	**<0.001**	**0.005**	**<0.001**	0.158	0.628	NA				
2006	**0.001**	**0.013**	**0.0001**	0.460	0.992	0.966	NA			
2010	0.975	0.999	0.957	0.753	**0.0001**	**0.002**	**0.001**	NA		
2011	0.999	1	0.999	0.130	**0**	**0.001**	**0.001**	0.992	NA	
2012	0.994	0.784	0.764	**<0.001**	**0**	**<0.001**	**0**	**0.016**	**0.023**	NA

Table 3.3: Correlation matrix of pair-wise comparisons resulting from ANOVA and Tukey's post-hoc test for δ^{13C}. Significant values ($p \leq 0.05$) are in bold. Values are symmetrical around the main diagonal, so only one set of values is shown.

Year	1996	2001	2002	2003	2004	2005	2006	2010	2011	2012
1996	NA									
2001	0.979	NA								
2002	1	0.815	NA							
2003	1	0.974	0.999	NA						
2004	**0**	**0**	**0**	**0**	NA					
2005	**<0.001**	**0**	**0**	**0**	0.999	NA				
2006	**0**	**0**	**0**	**0**	0.972	1	NA			
2010	**0.000**	**<0.001**	**<0.001**	**<0.001**	**0.000**	0.158	**0.000**	NA		
2011	**0.008**	**<0.001**	**<0.001**	**<0.001**	**0**	**0.004**	**0**	0.589	NA	
2012	0.994	0.224	0.986	0.851	**0**	**0**	**0**	**0**	**0**	NA

Implications of Inter-annual Variation on Population Structure Interpretations

In order to examine the effect that these significant temporal shifts have on population structure assignment, I applied a classification tree developed by Witteveen et al. (2009b) for the entire North Pacific humpback whale population to our data. Spatial isotopic variation in humpback whale populations across the entire North Pacific

observed in that study was approximately 2‰ for $\delta^{13}C$ and 2.5‰ for $\delta^{15}N$ (Witteveen *et al.,* 2009b). The classification tree, constructed with 2004 and 2005 data, had predicted 78% of group membership correctly for individual whales sampled by Witteveen *et al* (2009) belonging to the California/Oregon/Washington feeding group (our focal population).

I found that the high degree of temporal isotopic variability in our California samples significantly affected population structure interpretations based on the Witteveen *et al.* (2009) classification tree. The temporal isotopic variation in our samples (2.7 for $\delta^{15}N$ and 2.5 for $\delta^{13}C$) was slightly greater than the spatial variation observed across the North Pacific (Figs. 3.3 & 3.4). The 2004-2005 classification tree correctly predicted the feeding location of only 18% of the 1993-2012 CCE samples (Table 3.4). Excluding our 2004-2005 samples, correct classification dropped to 4% (Table 3.4). Most of our CCE samples from non-2004/2005 years were assigned to one of five other populations (Northern Gulf of Alaska (NGOA), Southeast Alaska (SEAK), Northern British Columbia (NBC), or the Central North Pacific (CENT)) (Table 3.4). The proportion of individuals from each year that were successfully assigned to the California population was then compared to the expected frequency for each year using a Chi squared test. Observed frequencies were significantly different than expected frequencies $\chi 2(10, N=291) = 121.6778$, $p = <0.001$.

Table 3.4: Results of geographic assignments based $\delta^{13}C$ and $\delta^{15}N$ isotopes of samples collected off California using a classification tree developed for the North Pacific by Witteveen et al. 2009a California data are from 11 different years during the period 1993-2010. . Only those samples classified as COW (California, Oregon & Washington) are correctly assigned. The other geographic strata are Northern Gulf of Alaska (NGOA), Southeast Alaska (SEAK), Northern British Columbia (NBC), and the Central North Pacific (CENT).

YEAR	COW	NGOA	SEAK	WEST	NBC	CENT	TOTAL	% correct
1993			1			2	3	0
1996			1		1	6	8	0
2001					1	7	8	0
2002	1	3	5			11	20	5
2003		4			1	10	15	0
2004	35	2	17	1	1	3	59	59
2005	5						5	100
2006	9		6		1		16	56
2010	2	1	18		1	3	25	8
2011		10	32	6	9	8	65	0
2012		8	10	10	9	30	67	0
Total	52	28	90	17	24	80	291	
% correct	18							

Ecosystem Shifts

Our study period is characterized by high degrees of oceanographic variability, with regards to both large-scale oceanographic forcing as well as localized upwelling events. Both the PDO and the MEI have been fluctuating at intervals of approximately 2 to 4 years for the last decade during which the NPGO also switched between positive and negative phases three times between 1993 and 2012 (PaCOOS, 2013). In response to these oceanographic and climactic conditions, abundance of potential humpback whale prey species also varied throughout the study period (PaCOOS, 2013). Anchovy and sardine abundances showed similar patterns of abundance, with positive abundance anomalies from 1993 through 2001 and 2003 through 2007 (Fig.3.11). Krill displayed an opposite pattern of abundance with positive anomalies from 2000 to 2003 and again from 2007 to 2012 (Fig. 3.11).

$\delta^{15}N$ ratios in humpback whales were found to be significantly correlated with sea surface temperature, sardine abundance and anchovy abundance. Sea surface temperature showed the strongest relationship, displaying a positive correlation with nitrogen ratios ($r^2 = .8994$, $p \leq 0.05$) (Fig. 3.6a). Anchovy and sardine abundance also showed significant positive correlations, though their relationships were slightly weaker (Anchovy: $r^2 = .6578$, $p \leq 0.05$; Sardine: $r^2 = 0.1996$, $p \leq 0.05$) (Fig. 3.6b and 3.6c). $\delta^{13}C$ was positively correlated with both sea surface temperature and sea surface height (proxy for ENSO) (SST: $r^2 = .4293$, $p \leq 0.05$; SSH: $r^2 = .3989$, $p \leq 0.05$) (Fig. 3.7). Both one-year and two-year lags were explored though neither were found to significantly improve correlations, so they were not considered further.

The best Generalized Additive Model, as assessed by AIC values, for humpback whale $\delta^{15}N$ included sea surface temperature and krill abundance (Fig. 3.8). Models that included anchovy, upwelling and sardine in addition to SST and krill also performed relatively well (within 4 AIC points). The relationship between $\delta^{15}N$ and SST was positive and generally linear, indicating that warmer years resulted in humpback whales feeding at higher trophic levels (Fig. 3.8). The relationship between krill and $\delta^{15}N$ is slightly more complex. The GAM function resulting from the model with SST and krill is non-linear and suggests that both low and high densities of krill result in enriched $\delta^{15}N$ in humpback whales (Fig. 3.8). However, this result is unlikely to be reflective of actual ecological processes and the model result is being driven almost exclusively by SST. Comparison of our best model (krill and SST) with a single variable SST model, shows that the AIC values are in fact within 5 points of one another. SST and krill abundance are moderately though not significantly correlated ($r^2 = 0.1238$, $p = 0.07$) which may also

drive some of the positive slope in the relationship between krill and $\delta^{15}N$ at high krill densities. Modeling $\delta^{15}N$ as a GAM function of the single variable krill indicated that, as expected, humpback whale $\delta^{15}N$ decreases monotonically with krill abundance (Fig. 3.9).

The best model for humpback whale $\delta^{13}C$ included year, anchovy, sardine and PDO (Fig. 3.10). $\delta^{13}C$ showed an overall positive trend during most of the study period, with the exception of 2012 (Fig. 3.10). $\delta^{13}C$ values were positively correlated with the abundance of anchovy in the ecosystem (Fig. 3.10). While sardine showed a negative relationship to $\delta^{13}C$, this does not accurately reflect the pattern evident from the annual data and, similarly to the relationship between $\delta^{15}N$ and krill, the explanatory power of sardine in the model is minimal (Fig. 3.10). This is confirmed by the difference of only two AIC points between the model containing sardine and the same model with sardine omitted. Lastly, PDO shows a non-linear relationship to $\delta^{13}C$ with an overall positive trend of increasing humpback whale $\delta^{13}C$ during positive phases of the PDO (Fig. 3.10).

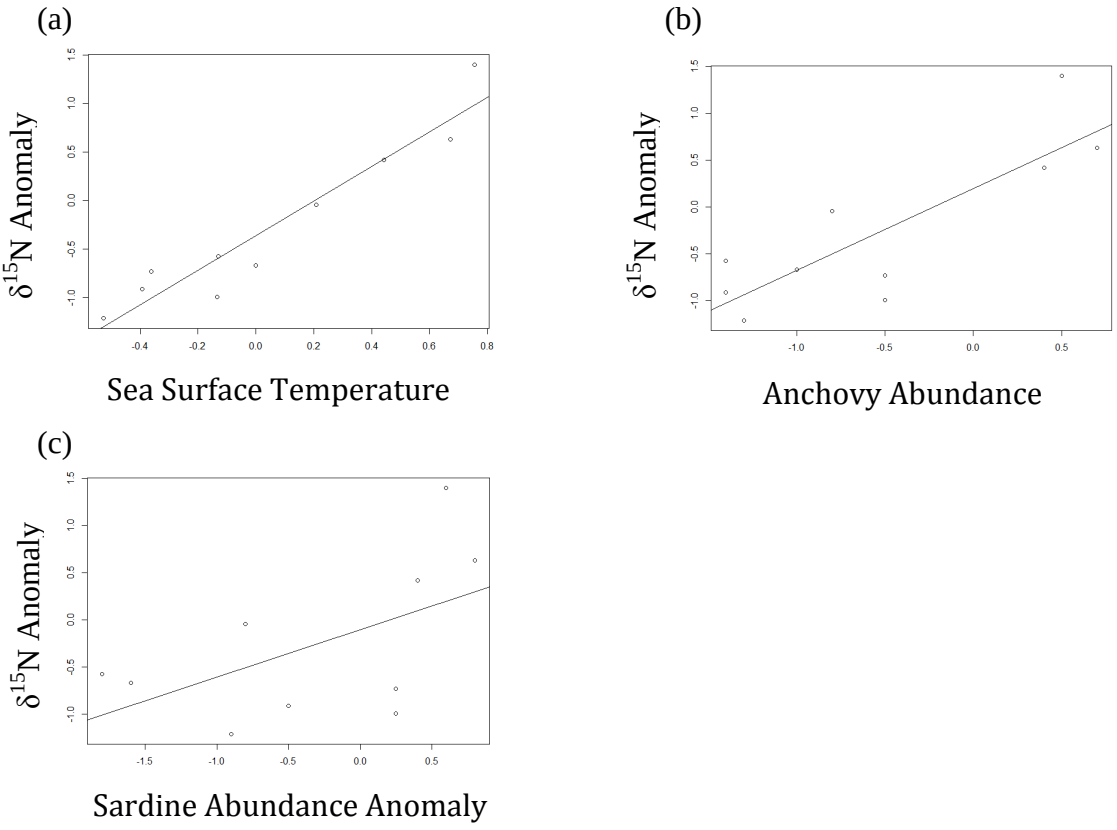

Fig. 3.6: Significant relationships found between humpback whale $\delta^{15}N$ and environmental variables. (a) humpback whale $\delta^{15}N$ and SST, (b) humpback whale $\delta^{15}N$ and anchovy abundance, and (c) humpback whale $\delta^{15}N$ and sardine abundance. Linear regression lines are shown.

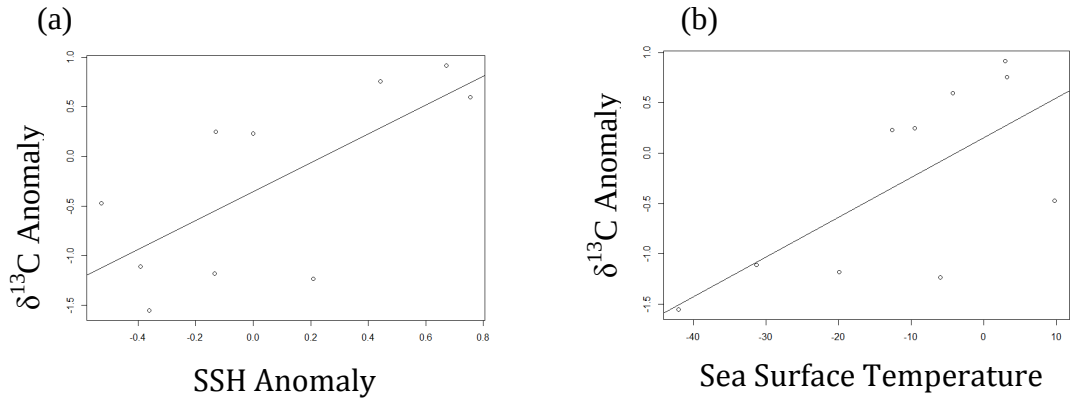

Fig.3.7: Significant relationships found between humpback whale $\delta^{13}C$ and environmental variables (a) humpback whale $\delta^{13}C$ and SST, (b) humpback whale $\delta^{13}C$ and SSH. Linear regression lines are shown.

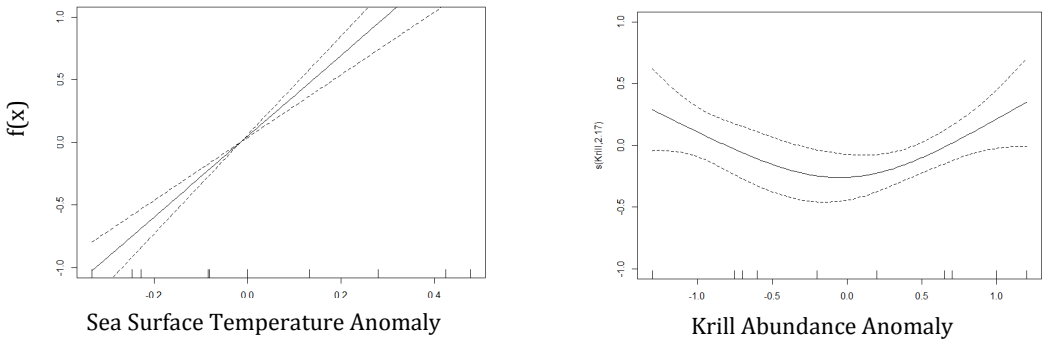

Fig. 3.8: Generalized additive model functions of humpback whale $\delta^{15}N$ in relation to sea surface temperature and krill abundance anomalies. Dashed lines are two standard error bars.

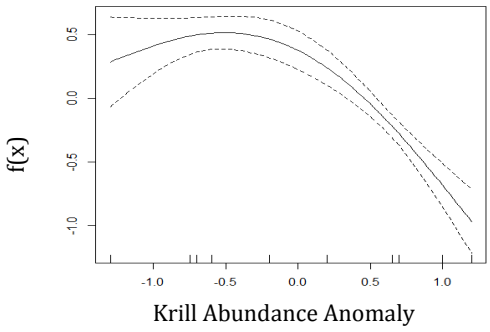

Fig. 3.9: Generalized additive model functions of humpback whale $\delta^{15}N$ in relation to krill abundance anomaly only. Dashed lines are two standard error bars

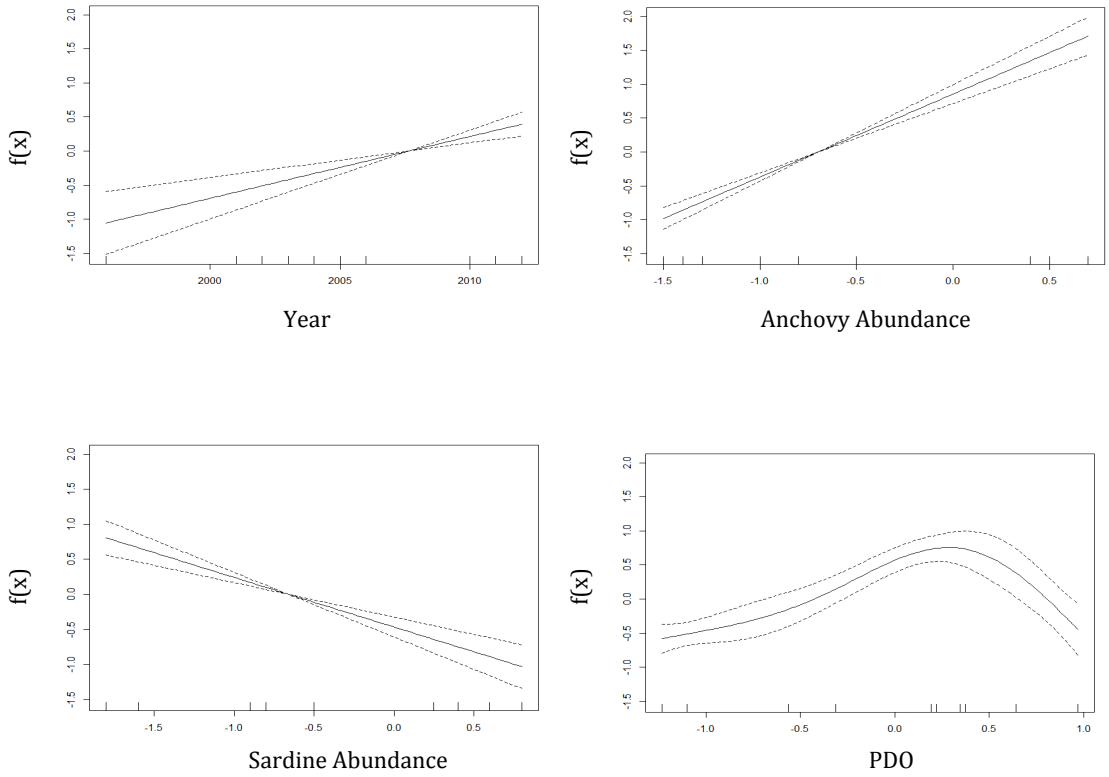

Fig. 3.10: Generalized additive model functions of humpback whale $\delta^{13}C$ in relation to year, anchovy abundance anomaly, sardine abundance anomaly and PDO. Dashed lines are two standard error bars.

Discussion

Determining population structure of large, highly-mobile top predators is a challenging task in the marine environment. Cetaceans, and mysticetes in particular, travel great distances both within and between seasonal habitats, and have diverse sets of needs in each seasonal habitat. They may shift distributions considerably in response to oceanographic conditions and these distributions can be difficult to detect on the temporal and spatial scales most relevant to the species (Forney *et al.*, 2000; Redfern *et al.*, 2006b). Defining habitat preferences and distribution patterns has significant implications for understanding population structure since habitat partitioning is recognized as a

mechanism promoting population differentiation (Geffen *et al.*, 2004). Characterizing these patterns improves management and conservation, allows for predictions of future distributions and densities, may provide insight into species responses in the face of climatic change, and allows for a more functional understanding of the species' ecological role in its local environment (Redfern *et al.*, 2006b).

Although diet preferences and habitat use measured through stable isotopes can add an additional level of understanding to investigations of population structure, it is first necessary to determine the temporal and spatial scales over which stables isotopes are most informative in a given system. There has been considerable interest in establishing stable isotope population signatures that could be used as markers for future studies of cetacean individuals. Since individuals sampled on breeding grounds are thought to maintain the signature of their feeding area, this would allow those individuals to be assigned to a specific feeding region without the need for a recapture event as is needed in both photographic and genetic studies of population structure (Witteveen *et al.*, 2009b). Studies to date have focused on determining whether spatially distinct populations have unique and identifiable isotopic ratios. These studies have utilized samples collected over short temporal scales, typically 1-3 years in length. They have found that stable isotopes have substantial promise as population markers and are especially useful for examining degrees of demographic connectivity (Born *et al.*, 2003; Swartz *et al.*, 2006; Querouil *et al.*, 2013).

While it appears that isotopic ratios can be a powerful tool for deciphering spatial or trophic differences between populations, I found significant temporal variability in population isotopic signatures with implications for population structure interpretation.

Our results suggest that in dynamic ecosystems, such as the California Current, the temporal scale over which isotope methods are applied and interpreted should be considered for both prey and predator samples. I recommend that the periodicity of major modes of oceanographic variability in the ecosystem be used as a guide for determining appropriate temporal scales. For other cetacean studies in the California Current, I recommend isotope-based classification be based on data collected within 2-3 years. If all populations of humpback whales across the North Pacific fluctuate with regards to their isotopic ratios to the same degree and with the same periodicity as the California Current population, it is possible that the problem posed by temporal variability could be negated. However, the inverse biological responses of the Gulf of Alaska and the CCS to climate forcing related to the PDO suggests that variance across the range of North Pacific humpbacks is unlikely to be temporally synchronized (Hare et al., 1999).

While the variable and dynamic nature of the CCS likely contributed to reduced temporal consistency in humpback whale isotope ratios, the shifting oceanographic conditions that occurred during our study period provide insight on ecosystem-wide responses to interannual variability in ocean-climate patterns. Our study period captured two major shifts in isotopic ratios of California humpback whales (Figs. 3.3 & 3.4). These shifts appear to be a result of a switch in their dominant prey type from krill to fish and back again. These prey switches reflect availability of prey in the system and changing oceanographic conditions (Fig. 3.11). Support for this hypothesis includes carbon and nitrogen isotope ratios of humpback whales, time series of prey abundances and time series of oceanographic conditions (Fig. 3.11).

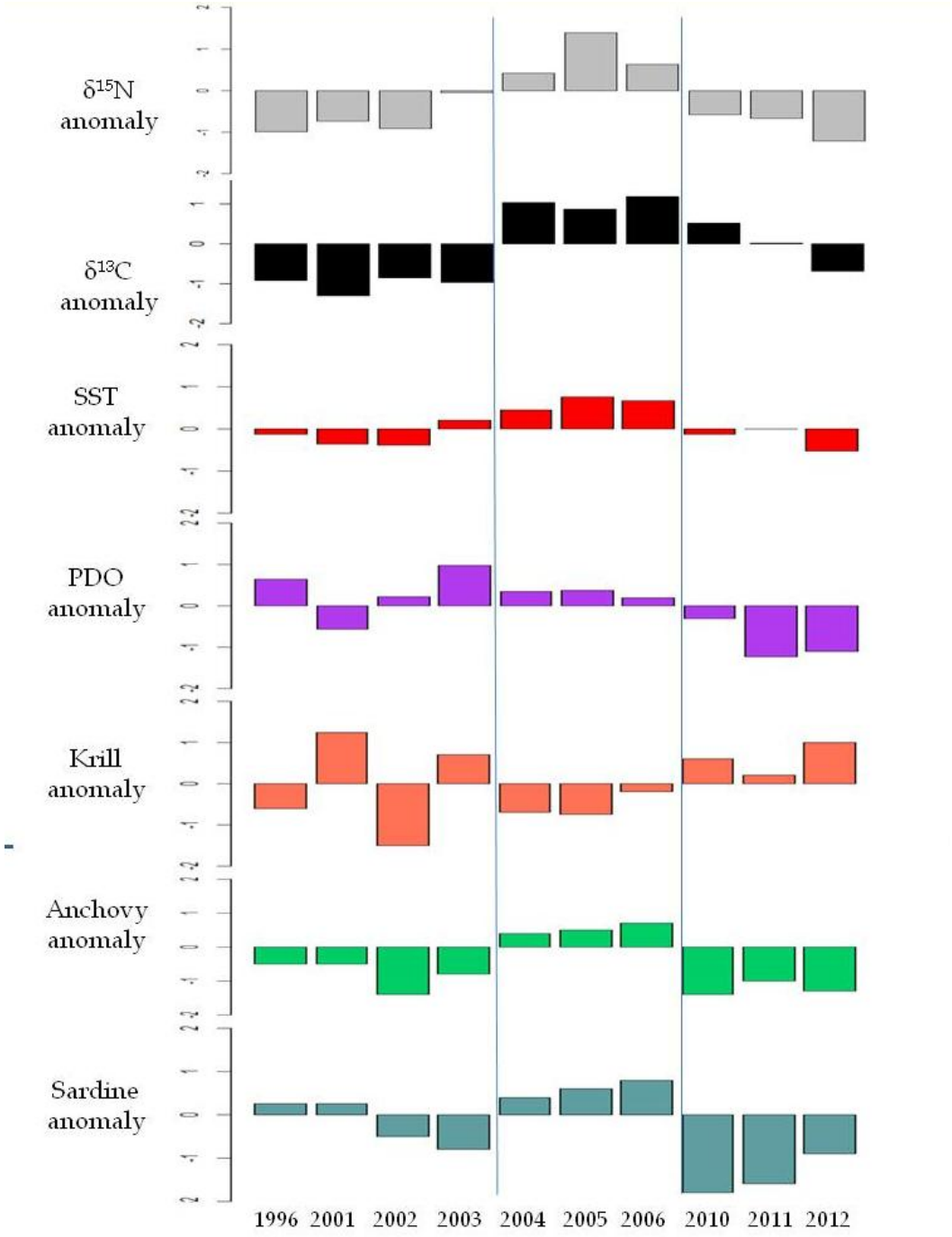

Fig. 3.11: Oceanographic indices, prey abundances and $\delta^{15}N$ and $\delta^{13}C$ ratios represented in standard deviation units. The timing of the two hypothesized prey shifts are indicated by the vertical gray lines.

Isotope Signatures

Isotope ratios suggest that the variance observed in humpback whale diet is indicative of a full trophic level shift, assuming no change in basal signatures in the food web. Both $\delta^{15}N$ and $\delta^{13}C$ vary by ~ 2‰ between the early, middle and late years of the study period (Fig. 3.11). Published values for krill (*Thysanoessa spinifera and Euphasusia pacifica*) $\delta^{15}N$ and $\delta^{13}C$ vary from values for anchovy and sardine also by approximately 2‰ (Table 3.5) (Becker *et al*., 2007; Brodeur *et al*., 2008; Miller *et al*., 2008; Miller *et al*., 2013; Sydeman *et al*., 1997). Given the importance of temporal variability, it would be preferable to have prey samples from all years of our study. However, it is difficult to obtain samples with the geographic coverage that is comparable to the humpback population range over the twenty-year time scale I examined. A review of the literature provided isotope values for prey samples collected in 1993, 1994, 1996-2002, 2007 and 2009 (Becker *et al*., 2007; Brodeur *et al*., 2008; Miller *et al*., 2008; Miller *et al*., 2013; Sydeman *et al*., 1997). All $\delta^{15}N$ and $\delta^{13}C$ values for krill were found to be comparable despite the different sampling years. This was also the case for sardine and anchovy, suggesting no change in basal signatures (their prey or phytoplankton). There was no overlap of carbon or nitrogen ratios between krill and these two forage fish species.

It is worth noting that 2010 has a unique combination of $\delta^{15}N$ and $\delta^{13}C$ signatures. While $\delta^{15}N$ is low, consistent with a krill-dominated diet, $\delta^{13}C$ is more enriched than would be expected from such a diet. While a krill-dominated diet it is consistent with prey availability and oceanographic indices that year, it is possible that the krill may have had a slightly altered $\delta^{13}C$ signature. $\delta^{13}C$ is usually indicative of location of forging, especially distance from shore (Post *et al.*, 2002). One possibility is that krill may have

had a more coastal distribution in the early part of 2010 feeding season. 2010 in the CCS was characterized by a transition in early spring from a brief El Niño period to La Niña (Bjorkstedt *et al.*, 2011). The winter was warmer than normal but cooler sea surface temperatures and increased upwelling resumed in June/July of 2010 (Bjorkstedt *et al.*, 2011). These conditions may have aggregated euphausiids closer to the shore. Additionally, the composition of copepods in 2010 was anomalously subtropical, similar to the composition of copepods observed in 2004-2006 (PaCOOS, 2011). This change in the type of prey available for euphausiids and the associated drop in lipid-content of the prey, may have impacted the resulting $\delta^{13}C$ signatures seen in humpback whales in 2010.

Table 3.5: Stable isotope ratios for potential prey items of humpback whales. Values are summarized from Becker *et al*., 2007; Brodeur *et al*., 2008; Miller *et al*., 2008; Miller *et al*., 2013; Sydeman *et al*., 1997 and encompass collection years of 1993,1994, 1996-2002, 2007 and 2009.

Prey Item	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)
Krill (*E. pacifica* & *T. spinifera)*	9 to 11	-21 to -19.5
Northern Anchovy (*E. mordax)*	13 to 14	-18 to -16.5
Pacific Sardine (*S. sagax)*	12 to 13	-19 to -16.5

Prey Availability

In 2010-2012, krill were present in high densities while both sardine and anchovy were at some of their lowest abundances throughout our study period (Fig. 3.11). From 2004-2006, the opposite pattern persisted with krill at low densities and anchovy and sardine were anomalously high. In 1996 and 2001-2003, sardine and anchovy were at or below their average abundances while krill was highly variable during this time period. However, the 2002 low krill abundance reported in the prey data used here might not

accurately reflect the abundance of krill in the CCS. While most species are considered to be well sampled, the midwater trawl surveys that our prey abundance data come from are not focused on the sampling of krill and may not fully reflect the true abundance of the species (PaCOOS, 2011) or the availability of krill to whales. Additionally, 2002 appears to have been a very high-density year for krill in the Southern CCS (Abraham & Sydeman, 2004). Cassin's auklets (*Ptychoramphus aleuticus*), a planktivorous seabird that feeds primarily on krill and nests on the Farallon Islands, had their most productive year compared to all 15 prior years the survey had been conducted (Abraham & Sydeman, 2004). Additionally, egg laying was initiated in February, the earliest start date on record (Abraham & Sydeman, 2004). From these multiple indicators, it would appear that krill was abundant in the CCS but the timing or geographic coverage of the SWFSC surveys may have been mismatched with the peak in krill abundance in the ecosystem.

Oceanographic Conditions

I explored the relationships between humpback whale isotope signatures and oceanographic conditions using regressions and GAMs. The variables most important for describing $\delta^{15}N$ were sea surface temperature and krill abundance. While temperature was the most important variable for $\delta^{15}N$, the effect of temperature on humpback isotope signatures is likely indirect and therefore the relationship was slightly improved with the addition of krill. Krill are typically more abundant in cooler conditions and are found adjacent to upwelling centers (Santora *et al.*, 2011). In contrast, the more synoptic indices such as PDO, NPGO and ENSO were not strongly correlated to humpback whale $\delta^{15}N$. The inclusion of both large-scale forcing indices (NPGO, PDO, ENSO) and local upwelling conditions (SST, CUI and SSH) in this study provides useful information for

future research that aims to connect top predator foraging and distribution with oceanographic and remotely sensed data. While numerous top-predator studies have explored relationships between large-scale indices and predator distributions, these findings suggest that local oceanographic data may be more explanatory.

The variables most correlated with $\delta^{13}C$ were PDO and anchovy and sardine abundance. While anchovy and sardine were more important in the model than PDO, the link between forage fish and humpback whale $\delta^{13}C$ is likely moderated by the strength and sign of the PDO. When the PDO is negative, southward transport in the CCS is stronger and the copepod community is dominated by subarctic species (Bi *et al.*, 2011). During these years, it appears that krill are present in greater densities and thus the dominant species in humpback whale diets, driving humpback whale $\delta^{13}C$ to be more depleted. When the PDO is positive, upwelling is often delayed and krill are less abundant (Bi *et al.*, 2011). However, anchovy and sardines have longer average life spans and may sustain greater population densities in these less productive years compared to krill, making anchovy and sardine available to humpback whales and subsequently increasing humpback whale $\delta^{13}C$.

While top predators are often cited as indicator species for ecosystem processes and conditions, it can be very difficult to determine the mechanistic links between predator foraging behavior and distribution, mid-trophic level prey dynamics, and oceanographic conditions (Hilty & Merenlender, 2000; Sydeman *et al.*, 2013). This study suggests that humpback whales are excellent indicators of ecosystem dynamics in the CCS. Humpback whales are highly flexible in their prey selectivity and must search out prey patches that are dense enough to support their metabolic needs. In doing so, their foraging

behavior is a synoptic result of conditions across the CCS. Data from California whaling stations from the 1920s through the late 1970s illustrate the integrative nature of humpback foraging. In the 1920s stomach contents were dominated by sardine (Clapham *et al.*, 1997; Rice, 1963). After the sardine crash in the late 1950s, stomach contents were predominantly anchovy (Clapham *et al.*, 1997; Rice, 1977).

I was surprised to find such a clean correlation between sea surface temperature and humpback whale $\delta^{15}N$ given the complexity of relationships between physical conditions and biological responses in such a dynamic environment. While numerous other studies have examined links between physical habitat and predator behavior, identifying very significant correlations over multiple years is less common (Zacharias & Roff, 2001). While variations in oceanographic conditions are occurring across daily and monthly time scales, our examination of annual and multi-year scales provides useful insight on the scales most relevant to predator foraging and population-level responses.

Previous research on the distribution and habitat use of cetaceans in the CCS compliment our findings here. Habitat models of humpback distribution reveal that whales were concentrated in a smaller area during the foraging season in both 2001 and 2008, in the same regions where persistent krill hotspots have been identified (Santora *et al.*, 2011; Barlow *et al.*, 2009). In contrast, in 2005, humpback whales were found to be more widely spread throughout the CCS extending into the California Bight (Barlow *et al.*, 2009). While an examination of geographic variability was beyond the scope of the present study, I hypothesize that the observed and predicted cetacean densities reported by Barlow et al. (2009) were the result of changes in location by humpback whales in order to exploit the most dominant prey resource in those years.

Since many studies have relied upon isotope data from past analyses to make current geographic assignments, assess trophic structure, or create isoscapes, this work provides an important cautionary tale that temporal variability should be considered before applying stable isotope analysis to marine mammal science. While long-term datasets of geographically coordinated prey and predator data are limited, I encourage future isotopic study design to emphasize temporal coverage and continuity. As this study has revealed, understanding of ecological processes operating at various trophic levels can be improved by examining these relationships under different oceanographic conditions.

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Chapter 3, in full, will be prepared for submission for publication of the material. Fleming, AH, Barlow, J, Calambokidis, J. The dissertation author was the primary investigator and author of this paper.

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Chapter 4.

Sources & magnitude of variability in cetacean stable isotope signatures: preservation considerations and implications for temporal interpretations

Abstract

Temporal investigations of cetacean diet, habitat use and movement patterns are necessary for understanding the ecology of these species and their adaptability in the face of environmental change. Stable isotope analysis is an excellent tool for these investigations. However, there are considerable methodological concerns that must be addressed before interpretations of temporal patterns can be made. This study examines two major issues in temporally-focused stable isotope studies: (1) effects of tissue preservative on stable isotope ratios and (2) variability of stable isotope ratios over time within an individual. I use skin samples and a baleen sample from humpback whales sampled in the California Current Ecosystem to investigate these methodological issues. I found that samples preserved in ethanol were significantly enriched in ^{13}C compared to frozen samples while samples stored in DMSO were significantly depleted in ^{13}C. ^{15}N was not significantly altered by preservative and resulting signatures were comparable across storage methods. $\delta^{13}C$ and $\delta^{15}N$ values were found to oscillate in a regular cyclical pattern along the length of the baleen plate, suggesting that stable isotope ratios in humpback whales change during migration. Skin tissue from repeatedly sampled individuals also showed that stable isotope ratios change over time periods of days to months. The intended use of any stable isotope data will dictate whether the magnitude of preservation-related effects and short-term temporal variability presented here should be

accounted for in future examinations of cetacean habitat use, migration, population structure and trophic ecology.

Introduction

Studies involving ecological applications of stable isotope analysis (SIA) have increased dramatically in the last decade especially for cetaceans (Newsome *et al.,* 2010). SIA has provided new insights on cetacean foraging ecology, migration, population structure, and habitat use. This information had previously been difficult to collect for cetaceans using traditional techniques such as direct observations or gut/scat content analysis given the complex life history patterns of marine mammals and their highly mobile pelagic distribution. Cetacean studies employing SIA have largely utilized the spatial patterns of isotopic signatures to examine these topics. However, interest in temporal studies on seasonal and interannual scales is growing as it enables investigation of changes in diet and habitat use and dietary specialization at the individual and population level (Newsome *et al*, 2010). Temporal considerations are critical for developing understanding of the dynamic processes that control cetacean foraging behavior, habitat use and ecological adaptability in light of natural climate variability and anthropogenic global warming.

There are a number of analytical considerations that must first be addressed in order to interpret temporal patterns in isotopic signatures of cetaceans. Methodological consistency is an inevitable challenge in long-term studies. For temporal studies using archived samples, variability introduced by sample preservation can be a significant concern since preservation methods change overtime, preservative type may have been selected for a different type of analysis, and samples may be stored for variable periods of

time. Additionally, natural variability within the individual animal over daily to seasonal scales remains largely unexplored for cetaceans. Short-term variability may result from temporal changes in ecology, tissue turnover or simply non-homogenous signatures across a tissue. Since temporal variability can be introduced through a variety of mechanisms, quantifying the magnitude of such variability at the level of the individual is necessary for interpretations of population level patterns and differences.

This chapter was partially motivated by the temporal study conducted in Chapter 3 and the associated methodological concerns that were raised during that research. Starting in 2004, all *M. novaeangliae* samples in the SWFSC archive were frozen while previously most samples were stored in DMSO with a small number of samples preserved in ethanol. Since this preservation switch occurred at the same time as one of the major observed ecological shifts in the California Current Ecosystem (CCE) humpback population, this raised the concern that a preservation signal might be driving the observed shift in $\delta^{13}C$ and $\delta^{15}N$.

Previous literature examining the effects of preservative on stable isotope analysis of carbon and nitrogen signatures in the tissues of different taxa has presented variable and sometimes conflicting findings (Todd *et al*., 1997; Hobson *et al.*, 1997; Marcoux *et al*., 2007; Barrow *et al*., 2008; Kurle & Worthy, 2002; Ruiz-Cooley *et al.*, 2011; Lesage *et al.*, 2010) (Table 4.1). Freezing or freeze-drying appear to be the best methods for sample preservation since neither method significantly alters carbon and nitrogen stable isotope signatures (Newsome *et al*, 2010). DMSO, ethanol and formalin were found to have significant effects on the tissue $\delta^{13}C$ and $\delta^{15}N$ signatures in some studies (Hobson *et al.*, 1997; Barrow *et al*., 2008). DMSO preservation commonly depleted $\delta^{13}C$ and $\delta^{15}N$,

however, lipid extraction of samples after preservation sometimes removes the effects of DMSO (Ruiz-Cooley *et al.*, 2011; Lesage *et al.*, 2010; Todd *et al*., 1997).

The humpback whale (*Megaptera novaeangliae)* samples available for the preceding study (Chapter 3) were either (1) frozen with no preservative, (2) preserved in DMSO or (3) preserved in ethanol. Most of the older samples were preserved in DMSO or ethanol while samples since 2004 were frozen. Since a preservative-related signature alteration could significantly impact the interpretation of any temporal signal in the data, I tested the effects of each of these preservatives on humpback whale skin isotopic signatures.

Tissue turnover rate and tissue homogeneity is another concern in stable isotope ecology. Especially for species that are highly migratory, acquiring a resample in order to estimate tissue turnover rates is difficult. To fully evaluate turnover rates, frequent resampling is needed which can only be conducted on captive animals. Most sampling of wild populations of cetaceans is done through biopsy sampling of skin tissue. The SWFSC tissue archive contained eleven occurrences of a repeat sampling event from eight different individual humpback whales. Inter-sampling intervals ranged from 1 day to 6 years. While this dataset does not allow for tissue turnover rates to be calculated, it provides a rare chance to examine stable isotope signature consistency within skin tissue from balaenopterids, which is absent from the literature.

Lastly, to better quantify isotopic signature variability in an individual humpback whale over time, we analyzed a baleen plate from its end to the point of insertion. Baleen plates contain keratinous layers generated over multiple years that record isotopic signatures from the time of formation. Baleen from right, gray, minke and bowhead

whales has been used previously to examine seasonal and multi-annual patterns in foraging (Schell & Saupe, 1993; Best & Schell, 1996; Mitani *et al.*, 2006; Caraveo-Patino *et al.*, 2007). The pattern of isotopic signatures along the length of the humpback baleen was examined to provide further information on isotopic signature variation rates and perspective for evaluating the degree of variability seen within the larger population. Though most samples collected from the larger population are skin tissue biopsy samples isotopic fractionation between diet and tissue is known to similar between skin and baleen (Borrell *et al*., 2012).

Table 4.1: Review of preservative studies focused on comparisons of DMSO, ethanol and freezing as storage methods. Significant effects of preservative storage are shown in bold.

Preservative Effects on Stable Isotope					
Publication	Type of tissue	Type of Preservative	Time in Preservative	$\delta^{15}N$	$\delta^{13}C$
Todd et al., 1997	humpback whale skin	DMSO	?	NA	lipid-extracted DMSO treated samples not significantly different than lipid-extracted non-DMSO treated samples
Marcoux et al., 2007	sperm whale skin	DMSO	2 weeks in DMSO after 2 year storage in ethanol	no sig difference between those left in ethanol ("control") and those soaked in DMSO for 2 weeks	no sig difference between those left in ethanol ("control") and those soaked in DMSO for 2 weeks
Hobson et al., 1997	sheep and quail	DMSO	8 weeks	**significant depletion compared to freeze-dried and powdered control (NO lipid-extraction)**	**significant depletion compared to freeze-dried and powdered control (NO lipid extraction)**
		Ethanol (70%)	8 weeks	no significant difference compared to freeze-dried and powdered control	no significant difference compared to freeze-dried and powdered control
Barrow et al., 2008	Green turtle skin	DMSO	1, 4, 15 and 30 days	**significantly depleted compared to control for both lipid extracted and non-lipid extracted samples (control= dried for 24h at 60°C)**	**significantly depleted compared to control for both lipid extracted and non-lipid extracted samples (control= dried for 24h at 60°C)**
		DMSO	60 days	no significant difference compared to control (control= dried for 24h at 60°C)	no significant difference compared to control (control= dried for 24h at 60°C)
		Ethanol (70%)	1, 4, 15, 30, and 60 days	no significant difference compared to control (dried for 24h at 60°C)	no significant difference compared to control (dried for 24h at 60°C)
		Freezing	1, 4, 15 and 30 days	no significant difference compared to control (dried for 24h at 60°C)	no significant difference compared to control (dried for 24h at 60°C)
		Freezing	60 days	significantly depleted compared to control (dried for 24h at 60°C)	significantly depleted compared to control (dried for 24h at 60°C)
Ruiz-Cooley et al. 2011	Squid muscle	DMSO	375	decreased except when LE	decreased except when LE
		Ethanol (70%)	375	no significant difference compared to control (-20C)	no significant difference compared to control (-20C)
Lesage et al., 2010	Bowhead	DMSO	1 yr	lipid-extracted DMSO samples not significantly different than lipid-extracted Frz	lipid-extracted DMSO samples not significantly different than lipid-extracted Frz
	Beluga	DMSO	1 yr	**enrichment even after LE compared to Frz NLE**	lipid-extracted DMSO samples not significantly different than lipid-extracted Frz
	Harbor Porpoise	DMSO	1 yr	**enrichment even after LE compared to Frz NLE**	lipid-extracted DMSO samples not significantly different than lipid-extracted Frz
	Balaenopterids	DMSO	1 yr	enrichment even after LE compared to Frz NLE	lipid-extracted DMSO samples not significantly different than lipid-extracted Frz

Methods

Variability between preservation methods

Eight individual whales were sampled during the 2011 feeding season between September and November in Monterey Bay. Each biopsy sample was split into three portions longitudinally in order to subsample all layers of skin. One subsample from each individual whale was then stored in ethanol, DMSO or was frozen in a -80^{o}C freezer for five to seven months. Though most of the samples analyzed in Chapter 3 were stored for much longer than 5-7 months, this is a longer period than has been examined in most other publications (Hobson *et al.*, 1997; Marcoux *et al*., 2007; Barrow *et al*., 2008) and therefore helps illuminate longer-term effects of storage mediums without delaying the progress of this study. Upon removal from the storage medium, lipids were extracted and samples were analyzed for δ^{13}C and δ^{15}N following the protocols detailed in Chapter 3. The effect of preservation method on δ^{13}C and δ^{15}N was evaluated using paired t-tests.

Variability within an individual

Baleen plates were acquired from the jawbone of a deceased humpback whale that was brought to the surface in the net of a fishing boat off the southern coast of Oregon. For the baleen used in this study, only the skull of the individual was brought to the surface and it was significantly decomposed. As a result, no information on the size of the individual, the year of death or its identity was available. However, humpback whales in this region are known to be part of the same feeding population that is found off California and was the source of the skin biopsy samples used in Chapter 3.

One baleen plate was selected and cleaned with ethanol. Using a dremmel tool, samples were taken along the length of the plate. Beginning at the proximal end of the

plate at the point of insertion in to the jaw, samples were collected every half centimeter. After the first 12 centimeters, samples were taken every centimeter. Baleen samples were not lipid extracted since keratin does not contain significant amounts of lipid (Newsome *et al*. 2010). With this procedural exception, analysis for $\delta^{13}C$ and $\delta^{15}N$ also followed the protocols detailed in Chapter 3.

Humpback whale skin samples were collected from individual whales in the California Current ecosystem between 34° and 42° latitude and 119° and 125° longitude from 1993-2012. Sampling only occurred from April to November when humpback whales are known to use this region for foraging. All samples were collected. Most samples were collected by biopsy, but a few samples of sloughed skin were obtained opportunistically with a dip net. All biopsy samples were acquired using a modified rifle or crossbow fitted with a hollow-tipped dart and included skin and a thin layer of blubber. For each sample, a unique sample number was assigned and the date, location and other observational data were recorded. Biopsy samples were frozen or stored in ethanol or DMSO upon collection.

Individual humback whales that were resampled during NOAA Southwest Fisheries Science Center marine mammal survey cruises or by Cascadia Research Collective from small boat platforms were identified to be the same individual by either genetic or photographic matching or both. Field sampling methods and analysis of skin tissue for stable isotope ratios followed protocols detailed in Chapter 3. The temporal consistency of $\delta^{13}C$ and $\delta^{15}N$ within an individual was assessed using a paired t-test. All statistical analyses were performed using R package version 2.15.2.

Results

Variability between preservation methods

Samples preserved in ethanol were significantly enriched in ^{13}C by 0.45‰ compared to frozen samples (SD = 0.25; range: -16.96 to -16.18), while samples stored in DMSO were significantly depleted in ^{13}C by ~0.41‰ (SD= 0.40; range:-18.38 to -16.94), (paired *t*-tests: ethanol vs. frozen t_s = 6.4, p < 0.001; DMSO vs. frozen t_s = -5.1, p=0.001) (Figs. 4.1 & 4.2). ^{15}N was not significantly altered by preservative and resulting signatures were comparable across storage methods (paired *t*-tests: ethanol vs. frozen t_s = 1.1, p=0.32; DMSO vs. frozen t_s = -0.2, p=0.78) (Figs. 4.1 & 4.2).

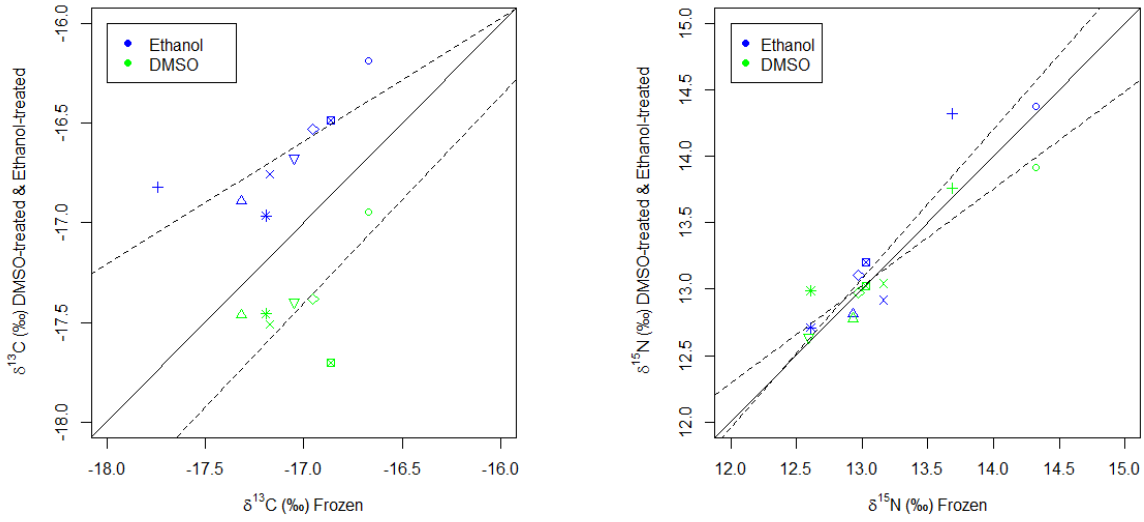

Fig. 4.1: Effects of DMSO and ethanol storage on humpback whale skin tissue (a) $\delta^{13}C$ and (b) $\delta^{15}N$ values. The solid line represents expected values if there is no difference between storage treatments.

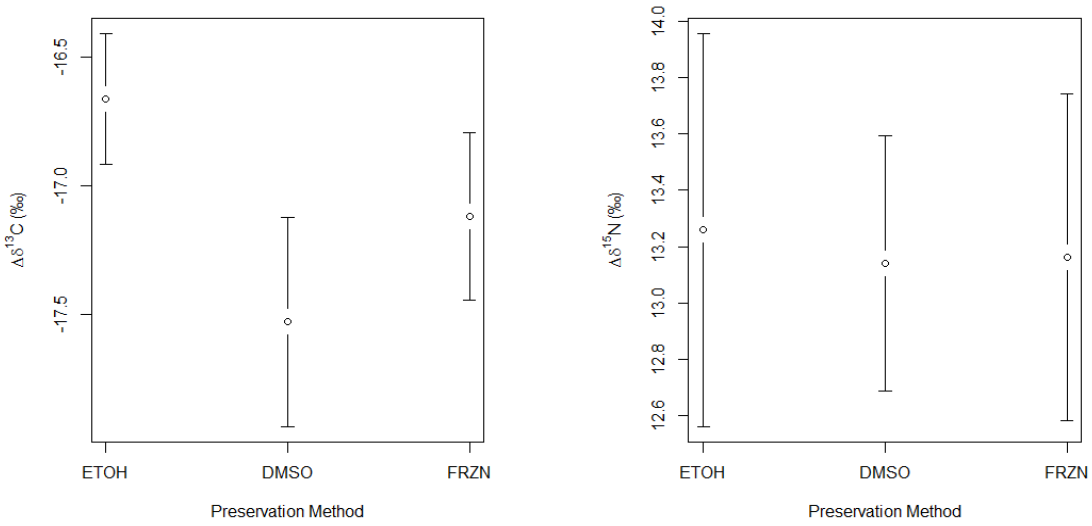

Fig. 4.2: Stable isotope values from humpback whale skin tissue under preservative solutions DMSO, ethanol (ETOH) or frozen (FRZR) for both C and N.

Variability within an individual

$\delta^{13}C$ and $\delta^{15}N$ values were found to oscillate in a regular cyclical pattern along the length of the baleen plate (Fig. 4.3). The pattern of the variations suggests that there is a strong migratory signal reflecting movements between breeding and feeding grounds, as has been found in other baleen whale species (Best & Schell, 1996, Hobson & Schell, 1998, Lee *et al.*, 2005). The plate contained about two and a half oscillations in $\delta^{13}C$ and $\delta^{15}N$. Assuming these oscillations reflect migration on annual time scales, the growth of humpback baleen can be estimated to be 20cm/year, which falls between that estimated for minke (~13cm/yr) and bowhead whales (19-25cm/yr) growth rate (Schell & Saupe, 1993; Mitani *et al.*, 2006). This is the first estimate of growth for humpback whale baleen. The magnitude of variability along the baleen plate was approximately 3.48‰ in ^{15}N and 1.59‰ in ^{13}C over the two and a half years of growth contained in the plate.

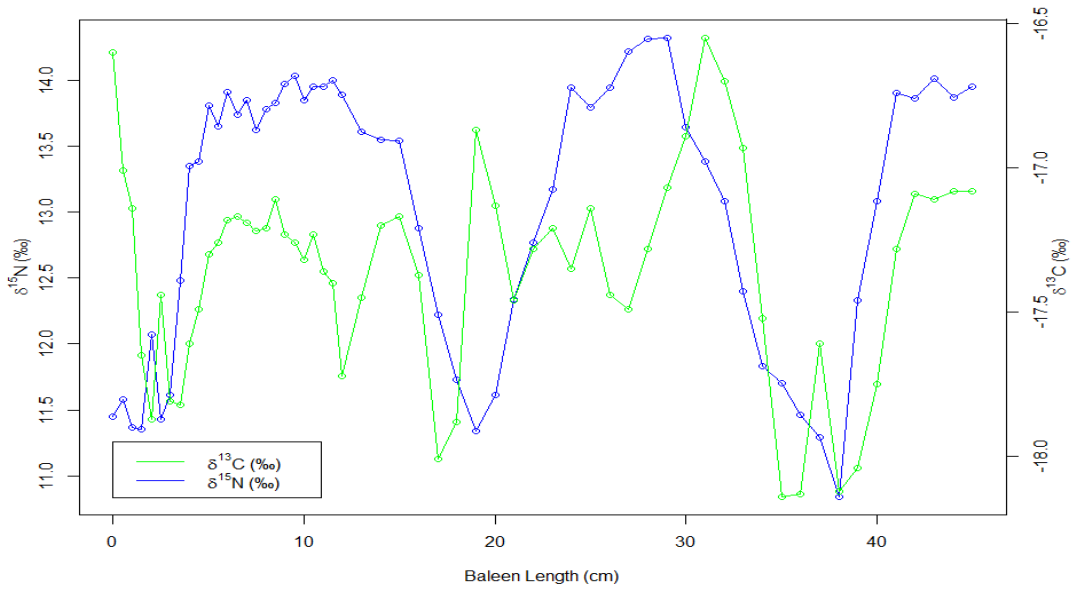

Fig. 4.3: $\delta^{13}C$ and $\delta^{15}N$ values along the length of a single humpback whale baleen plate. The most recently formed baleen is on the left.

Individuals resampled on the feeding grounds showed a range of shifts in isotopic signature (Fig. 4.3). Some individuals showed almost no change in either $\delta^{13}C$ or $\delta^{15}N$ while other resampling events resulted in a difference of 0.8‰ in $\delta^{13}C$ and 1.0‰ in $\delta^{15}N$. Though most of the larger shifts occurred over longer time periods of approximately 3-4 months, changes in both $\delta^{13}C$ and $\delta^{15}N$ within just 5 days of the first sampling event were 0.05 - 0.3‰ for ^{15}N and 0.03 - 0.4‰ for ^{13}C (Fig. 4.4). Despite these individual ranges, when examined as a group the mean values of the resampled individuals did not differ significantly between the first and second sampling events for either $\delta^{13}C$ or $\delta^{15}N$ (paired *t*-tests: $\delta^{13}C$ *ts*= -0.5791, p=0.57; $\delta^{15}N$ *ts*=0.6108, p=0.55).

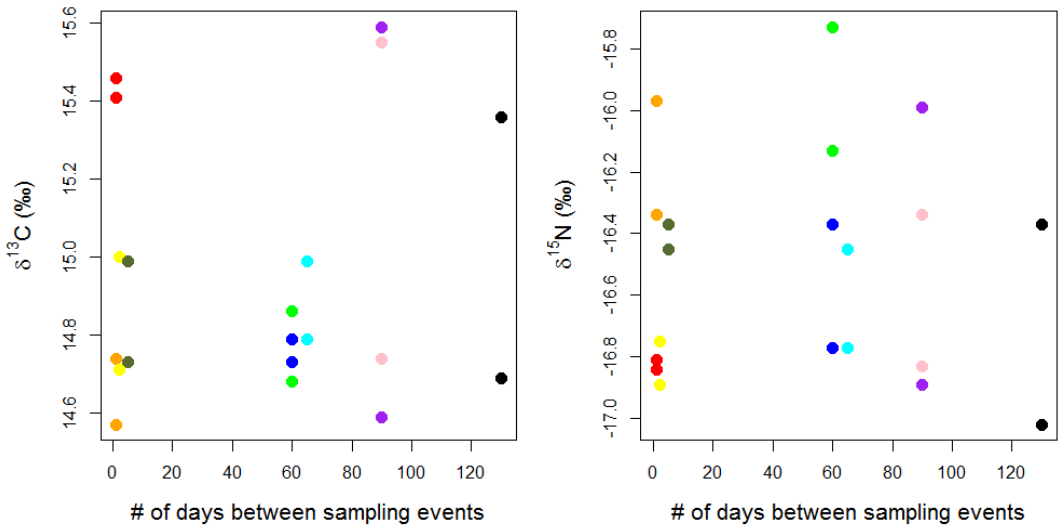

Fig. 4.4: $\delta^{13}C$ and $\delta^{15}N$ values of resampled individual whales. Each individual whale is represented by a unique color.

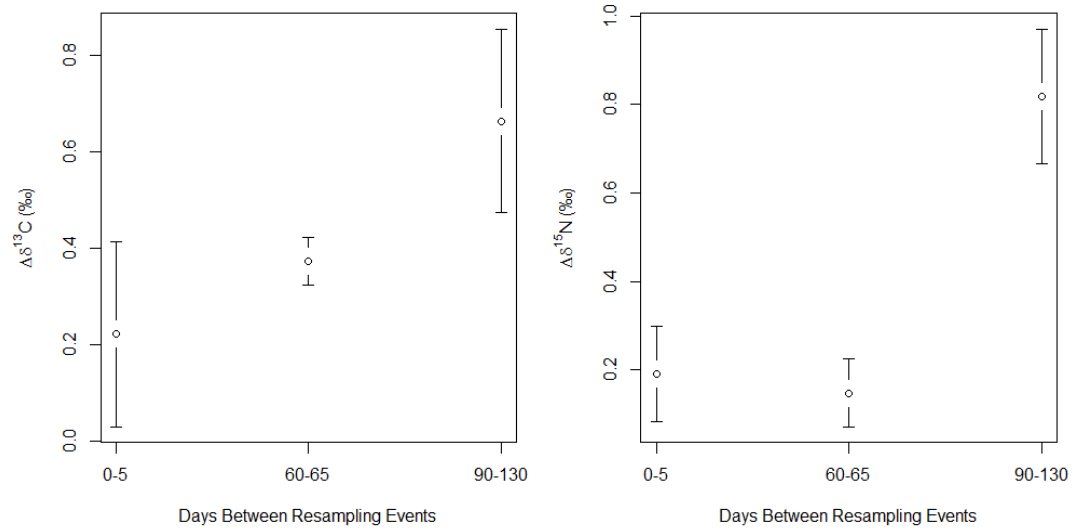

Fig.4.5: Average change in humpback whale skin $\delta^{13}C$ and $\delta^{15}N$ values between resampling events. Resampling events were grouped by sampling interval.

Discussion

Variability between preservation methods

The results of the preservation test confirm that freezing is the preferred method for storing humpback whale samples for SIA. Both DMSO and ethanol had significant

effects on $\delta^{13}C$ signatures. This conflicts slightly with the findings from most previous studies (Table 4.1). While many studies have found that DMSO depletes isotope ratios by 4.5-7‰ most research to-date suggests that lipid extraction removes the effect of DMSO and restores isotope ratios so that samples are comparable to frozen control samples (Ruiz-Cooley *et al.*, 2011; Lesage *et al.*, 2010; Todd *et al.*, 1997). Lipid extraction of the humpback whale skin tissue samples used in this study did not fully restore $\delta^{13}C$ values. Additionally, most studies on cetaceans have not found ethanol to cause significant alterations to carbon or nitrogen isotope signatures, in contrast to the findings presented here (Hobson *et al.*, 1997; Barrow *et al.*, 2008; Ruiz-Cooley *et al.*, 2011). These results indicate that numerous analytical concerns, including the effects of preservation and the impacts of lipid extraction, are very species- and tissue-dependent.

While the effects of preservation were significant, the magnitude of the effect may also be valuable to consider when interpreting these findings. The magnitude of change in $\delta^{13}C$ observed here does not invalidate the ecological conclusions made in Chapter 3. Humpback whales sampled from 1993-2003 that were included in Chapter 3 had lower $\delta^{13}C$ values than humpback whales sampled from 2004-2012. Either DMSO preservation or feeding at lower trophic levels could lead to a depletion in $\delta^{13}C$. However, the shift seen in the CCE humpback whales was over 1‰ in $\delta^{13}C$ between 2003 and 2004 which is significantly greater than the ~0.4‰ depletion caused by storage in DMSO. It should be noted that all of the 1993-2003 samples utilized in Chapter 3 were stored for at least 8 years before being analyzed. There is the possibility that longer storage in DMSO may have caused more depletion than we observed here but a longer test period was not within the scope of this study. While this preservation effect likely

contributed to the observed shift seen between pre-2003 samples and post-2004 samples, a trophic switch remains the most parsimonious explanation for the magnitude of observed change.

The intended use of any stable isotope data will dictate whether preservation-related effects can be appropriately accounted for, especially in archival studies. The magnitude of change in $\delta^{13}C$ that resulted from storage in DMSO and ethanol, ~0.4‰, is less than the 1-3‰ enrichment in $\delta^{13}C$ typically associated with trophic level shifts. Other applications of stable isotope analysis for studies of cetaceans include population structure interpretations, also discussed in Chapter 3. Differences between humpback whale feeding groups in the North Pacific range from 0.3‰ to 2.5‰ for both $\delta^{13}C$ and $\delta^{15}N$ (Witteveen *et al.*, 2009b). If samples collected from one population were predominantly stored in DMSO and compared to samples from another population that had been frozen, the ~0.4‰ preservative effect observed here could inhibit accurate population assignment. Similarly, differences between breeding grounds of North Pacific humpback whales ranged from 0-2‰ for $\delta^{13}C$ and $\delta^{15}N$ and therefore population assignments across breeding grounds may be complicated by preservative effects.

While the exact mechanism for DMSO-associated depletion of $\delta^{13}C$ is still not fully understood, there are a few possible explanations for the difference between the findings presented here and in other studies. First, the impact of DMSO and lipid extraction may be species-specific. Humpback whales have a lower C:N ratio than fin whales and minke whales, two closely related species that have also been the subjects of preservative comparisons for stable isotope analysis (Ryan *et al.*, 2012). This lower C:N ratio is indicative of a lower lipid content in humpback whale skin (McConnaughey *et al.*,

1979). Lipids are enriched in ^{12}C compared to bulk proteins which decreases bulk tissue $^{13}C/^{12}C$ and therefore $\delta^{13}C$. DMSO typically further depletes $\delta^{13}C$. This effect is then reversed by the removal of lipids which also removes ^{12}C, restoring $\delta^{13}C$ values. The differential response of humpback whale tissue to DMSO preservation and lipid extraction as compared to the other investigated species, suggests that the effect of DMSO and lipid removal may be dependent on the lipid content of the tissue.

Additionally, the solvent used to extract lipids can vary by laboratory. Petroleum ether was used in this study while chloroform and methanol are most frequently used. Petroleum ether has been suggested to be the better solvent as it may remove less non-lipid material (Dobush *et al*., 1985). $\delta^{13}C$ signatures are sourced from protein, carbohydrate and lipid components of the diet while $\delta^{15}N$ is largely from protein (McConnaughey *et al*., 1979). If less non-lipid material was removed from our samples by petroleum ether as compared to chloroform and methanol in other studies, our resulting $\delta^{13}C$ signature may be reflecting a slightly different composition of remaining bulk tissue. Lastly, the results of this preservation comparison further confirm the conclusions of many previous studies. There is a high degree of species-specific and tissue-specific variability that is difficult to predict even with preservation data from closely related species. When compounded by slight variations in laboratory methods, there are considerable analytical issues that should be addressed by each application of SIA before conclusions are drawn.

In most previous studies of mammalian $\delta^{13}C$ and $\delta^{15}N$ values, ethanol had not been found to affect either ^{13}C or ^{15}N isotope ratios (Hobson *et al.*, 1997; Barrow *et al*., 2008; Ruiz-Cooley *et al.*, 2011). However, numerous studies of other taxa including fish,

squid and octopus reported increases in $\delta^{13}C$ and $\delta^{15}N$ after storage in ethanol (Sweeting *et al*., 2004; Kaehler and Pakhomov, 2001). The magnitude of change found in the present study is smaller than that reported for squid, fish and octopus so the significance finding may be influenced by our small sample size. The enrichment in $\delta^{13}C$ observed in ethanol-preserved samples is likely a result of the removal of isotopically light lipids (over that achieved with our standard lipid extraction) which results in increased $\delta^{13}C$ in the bulk tissue (Sweeting *et al*., 2004; Kaehler and Pakhomov, 2001; Carabel *et al*., 2009). While the degree of alteration caused by ethanol is similar to that caused by DMSO, the direction of change caused by these two preservatives is opposite in size, emphasizing the variable ways in which preservative may influence $\delta^{13}C$ and $\delta^{15}N$ values in humpback whale skin. The significant impact of ethanol in this study is particularly noteworthy since previous studies concluded that this preservative did not alter isotopic ratios.

Variability within an individual

Analysis of the baleen plate revealed a high degree of temporal variability in $\delta^{13}C$ and $\delta^{15}N$ within an individual humpback whale (Fig.4.2). The strong cyclical nature of this variability indicates that $\delta^{13}C$ and $\delta^{15}N$ are reflecting periods of feeding and fasting during annual migration. These same oscillations have been observed in southern right whales (*Eubalaena australis)*, bowhead whales (*Balaena mysticetus)* and gray whales (*Eschrichtius robustus*) (Best & Schell, 1996; Caraveo-Patino *et al*., 2007; Schell & Saupe, 1993). These studies have suggested that fasting leads to elevated $\delta^{15}N$ levels since animals would be feeding on $\delta^{15}N$-enriched body proteins while metabolizing their own energy stores. However, this has not been observed in all studies that examined

stable isotope ratios of migratory species (Hobson & Schell, 1998; Ben-David *et* al., 1999; Williams *et* al., 2007). Humpback whales are capital breeders that make annual long-distance migrations to breeding grounds that are characterized by warm, shallow, low-nutrient oceanographic conditions. They must depend on lipid stores in the form of blubber in order to maintain their breeding and migratory activities. The behavior and natural history of this species make it much more likely that they are catabolizing their lipid stores than their protein stores. Since lipids are depleted in ^{13}C this metabolic process would result in lower $\delta^{13}C$ bulk tissue values. This would suggest that the valleys in $\delta^{13}C$ in figure 2 are reflecting time on breeding grounds. These valleys largely overlap with valleys in $\delta^{15}N$. While only having one sample makes it difficult to draw conclusions, there appears to be a slight lag in $\delta^{15}N$ behind $\delta^{13}C$ along the baleen plate. This may reflect preferential metabolism of $\delta^{13}C$.

The breeding grounds of humpback whales that feed off California and Oregon are primarily located off the Pacific coast of Central America. These waters are depleted in $\delta^{15}N$ compared to the California Current (Ruiz-Cooley & Gerrodette, 2012; Somes *et al.*, 2010). While the influence of background environmental isotope ratios on baleen signatures remains unknown, the drop in $\delta^{15}N$ in the baleen would match the drop in environmental $\delta^{15}N$ as the individual migrated south to the breeding grounds. This would only occur if humpback whales continue to feed during their southbound migration, which is currently not thought to happen. Additionally, the $\delta^{15}N$ plateaus for longer periods when $\delta^{15}N$ is higher. This would be most expected on the feeding grounds since individuals are likely to remain there for longer periods of time (~6 months) than on the breeding ground (~2-4 months) and are consistently feeding. It is unclear whether baleen

growth rate is constant or variable but if baleen growth rate increased during periods of foraging when the animal has more resources and organic material for anabolism of body tissues, a greater amount of baleen tissue would be expected to show a constant signal, similar to the observed plateaus. Lastly, the elevated values of $\delta^{15}N$ and $\delta^{13}C$ that are observed in the peaks in the baleen oscillations match those seen in humpback whale skin tissue on the California/Oregon feeding ground (see chapter 3) further suggesting that the peaks correspond to a feeding ground signature. While isotopic fractionation between diet and tissue is known to vary with tissue type, the fractionation between skin and baleen is minimal and a similar feeding ground signature would be expected in both the skin and baleen (Borrell *et al*., 2012).

The magnitude of change observed in the baleen $\delta^{13}C$ and $\delta^{15}N$ values within one individual in one year is comparable to the observed difference between distinct feeding populations in the North Pacific, with implications for population structure interpretations (Witteveen *et al.*, 2009a, Witteveen *et al.*, 2009b). Interestingly, no significant difference in skin isotopic signatures was seen in individual humpback whales that were sampled on both the feeding and breeding grounds while the baleen results here suggests that a substantial difference may exist between breeding and feeding ground signatures (Witteveen *et al.*, 2009b). Since the continuity of an individual's isotopic signature has important ramifications for interpreting migratory destinations and population structure, analysis of additional baleen plates could be advantageous. Additionally, the 3‰ change in $\delta^{15}N$ and 2‰ change in $\delta^{13}C$ has implications for assessing seasonal and interannual changes in diet since this is equal or greater than the shift observed due to trophic level enrichment (Peterson & Fry, 1987). Though this is only one sample of humpback whale

baleen, it has provided insight into the degree of variability seen within an individual over a multi-year time frame and new possibilities for the interpretation of metabolic processes governing isotopic signatures in a migratory species.

In addition to the information gained from the baleen, the repeatedly sampled individuals provide further insight into intra-individual isotopic variation. While tissue turnover time can't easily be calculated in wild populations, these repeat samples provide a first chance to examine the consistency of isotopic signatures over short time periods. The results of the t-test indicate that, when pooled, the first and second samples from each individual are not significantly different from each other. However, for each individual the degree of change observed is larger than would be expected on such short time scales (Fig. 4.4). Especially for ^{13}C, the differences observed on daily time scales are large compared to the precision of the analysis (0.2-0.4‰ difference between re-sampling events vs. 0.05‰ for analytical error of laboratory standards). Therefore, a methodological issue is unlikely to be the major culprit of this variability. However, each sample was likely taken from a slightly different part or side of the body, suggesting that skin tissue isotope signatures may not be completely homogenous (Friedlaender, A., *pers. comm.*) Variability in both $\delta^{13}C$ and $\delta^{15}N$ did increase with increasing time between sampling events. The larger observed changes in isotopic signatures over slightly longer time frames (60-130+ days) are more likely to reflect some degree of change in diet signature or location in addition to tissue homogeneity differences. Humpback whales can travel across large geographic areas of the feeding ground in relatively short periods of time which may cause repeat sampling signals to reflect slightly different habitats within the CCE.

The findings of this study clarify some of the analytical concerns involved in long-term isotopic studies which I hope will promote additional temporally-focused investigations. My results support freezing as a preservation method for samples used for SIA. However, given the importance of temporal investigations, samples stored in alternative preservatives may still be informative depending on the questions being asked and the scale of isotopic shifts involved. Given the disparity between my results here and previous studies, in terms of the effect of DMSO on $\delta^{13}C$ remaining even after lipid-extraction, I would caution against the use of a general cetacean correction factor. This study supports the conclusions of other studies that effects of preservative and lipid-extraction are highly species- and tissue-specific.

Both the baleen and skin samples from repeatedly-sampled individuals reveal the high degree of variability in isotopic signatures that can occur within an individual on intrannual time scales. The magnitude of this short-term temporal variability should be considered in future applications of SIA to examinations of cetacean habitat use, migration, population structure and trophic ecology.

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Chapter 5.

Refining understanding of population distribution: modeling of Dall's porpoise habitat preferences using acoustic detections

Abstract

A fundamental step in modeling species ecology is the collection of accurate population size and distribution data. This is especially challenging for less conspicuous or deep-diving cetaceans. Dall's porpoise, a common, vocally-active cetacean found in cool waters of the North Pacific, is nearly impossible to sight in rough seas due to its small body and group size. Additionally, the extent of Dall's porpoise southern range fluctuates significantly in response to oceanographic variability in the California Current. These factors have led to questions about habitat requirements and statistical power in abundance analyses for the species. To address this, passive acoustic detections of Dall's porpoise during a 2008 NOAA marine mammal survey of the California Current were used to investigate the distribution and to build predictive habitat models for this species. Distribution was examined in relation to depth, slope, aspect, sea-surface salinity and temperature, chlorophyll, mixed layer depth and distance from shore. Acoustic methods significantly increased detection frequency and geographic areas previously void of visual detections contained numerous acoustic detections. Generalized Additive Models (GAMs) built with acoustic data were compared to visual-based models. Acoustic models confirmed findings from visual models and expanded upon our current understanding of Dall's porpoise habitat preferences. Dall's porpoise distribution was best predicted by a combination of bathymetric and biological variables including sea-surface temperature, mixed layer depth and slope. It is clear that the combination of both

acoustic and visual methods provide a more accurate baseline for future predictions and investigations of temporal and spatial variability in Dall's porpoise distribution.

Introduction

Numerous top predators in the marine environment display shifts in population distribution and range in response to oceanographic conditions that vary across seasonal, interannual and decadal scales. This temporal variability in distribution and density patterns can create challenges in discerning habitat preferences of a population. However, assessing the oceanographic processes and trophic relationships that determine these patterns is critical for understanding species ecology and designing appropriate monitoring and management plans (Bailey *et al*., 2009; Azzellino *et al*, 2012). Such a dynamic understanding allows for predictions of future distributions and densities, may provide insight into species responses in the face of climatic change, and allows for a more functional understanding of the species' ecological role in its local environment (Redfern *et al.*, 2006b). Additionally, defining habitat preferences and distribution patterns has significant implications for understanding population structure since habitat partitioning is recognized as a mechanism promoting population differentiation (Geffen *et al.*, 2004).

Habitat modeling is an increasingly popular tool for examining the biotic and abiotic variables that best characterize observed predator distribution patterns (Reilly, 1990; Yen *et al.*, 2005; Redfern *et al.*, 2006). These models allow finer scale resolution of predator density than line-transect survey estimates because they allow for interpolations to be made between transect lines, providing estimates of density across the entire habitat

(Forney *et al*., 2012). For species that are rare or have behaviors that bias survey-based estimates, habitat models can substantially improve resolution of distribution and density (Rogers *et* al., 2013).

Dall's porpoise, *Phocoenoides* dalli, is a common cetacean found in cool temperate waters of the North Pacific between 32°N and ~63°N (Jefferson, 1988). The species habitat requirements are poorly understood because its small body and group size make visual sightings nearly impossible in rough seas (Barlow, 2010). Dall's porpoise are deep divers with diverse diets that include both mesopelagic and epipelagic species including squids, Pacific hake, Pacific herring, northern anchovy, and juvenile rockfish (Jefferson *et al*., 1988). It remains debated whether this species primarily feeds nocturnally or more continuously through the day. They do not exhibit a population wide migratory pattern but do move closer inshore and shift their distribution to the south during cooler months (Jefferson *et al*., 1988).

Previous research in the California Current found Dall's porpoise abundance to be inversely related to sea surface temperature (Forney, 2000). However, that study concluded that the species-environment relationship may not have been fully captured by the analyses (Forney, 2000). Previous abundance estimates for the species based on line-transect studies have varied significantly between warmer and cooler years since a different proportion of the population is present in the survey area. Dall's porpoise also display a unique response to ships, approaching vessels to bowride, which violates a major assumption of line-transect abundance estimation methods (Jefferson *et al*., 1988). All of these behavioral traits have made abundance estimates less accurate for the species, and no trend information currently exists.

Most information on Dall's porpoise distribution to date has been collected through visual observations during scientific surveys. Though they are effective, visual surveys are hindered by inclement weather, rough seas and missed sightings, and are limited to observing animals only at the sea surface (Roch *et al.*, 2007). Sightings of Dall's porpoise are lower in higher sea states and any sightings made in conditions over Beaufort 2 are often of animals that have reacted to the presence of the ship. These sightings violate line-transect method assumptions and are therefore not included in density estimates. This limitation severely decreases the survey effort available for detecting Dall's porpoise and for determining habitat-occupancy patterns. For example, during the four-month cruise that provided the data for this study, less than 2% of survey effort was in Beaufort sea-state 2 or below.

Passive acoustic surveys provide an alternative means of detecting vocal animals underwater, at greater distances, and in poor weather conditions. This technology is ideally suited to studying Dall's porpoise because this species vocalizes frequently, allowing reliable acoustic presence/absence data to be gathered (Barlow, Forney, 2007). Dall's porpoise produce narrow-band high frequency clicks (Kyhn *et* al., 2013). These echolocation clicks are used as a biosonar system for locating and classifying prey within short distance ranges. Source levels of Dall's porpoise clicks have been measured at 187±7dB re 1 µPa (peak-peak) and measurements of mean centroid frequency have ranged from 120±4 kHz to 137±3 kHz (Kyhn *et* al., 2013; Basset *et al., in prep*). The high-frequency nature of their vocalizations and high-directionality results in high attenuation or transmission loss and estimates of detection range are on the order of <500m (Kyhn *et* al., 2013). Although the addition of acoustics allows for more

continuous survey effort, the nature of these calls does restrict the effective area surveyed.

The technology required to record such high-frequency vocalizations was not accessible for our field operations until 2008 and little other research has been conducted on Dall's porpoise vocalizations. However, during this cruise, recordings from Dall's porpoise single-species groups were made which allowed for more thorough measurements of click characteristics revealing at least two characteristic click types that allow Dall's porpoise to be identified acoustically to the species level (Bassett *et al.*, *in prep*).

While habitat modeling based on visual surveys has proved to be a highly effective management and conservation tool, models based on acoustic data have only recently been developed (Rogers *et al.*, 2013; Booth *et al.*, 2013). Both methods detect a portion of the true presence of animals in an area. Visual methods are dependent on animals being at the surface while acoustic methods depend upon animals vocalizing. Though neither of these methods can capture the full suite of individuals in an area, combining distribution information gathered from acoustic data with that gathered from visual observations would form a more robust picture of this species' distribution and habitat preferences. Here, we develop the first models of Dall's porpoise encounter rates based on acoustic detections and compare them to models built with visual data. We limit our models to encounter rate, rather than density, because we are currently unable to determine the number of individuals vocalizing in an acoustic detection event. This study provides an important initial step towards future models that will integrate both sources of data, visual and acoustic, into a single model. Studying this population of Dall's

porpoise through a different metric should enable a more holistic assessment of how their distribution and population structure relates to prey ecology, oceanographic, and bathymetric variables in their habitat. This information will be crucial to predicting future distribution patterns and managing this protected species at the population level.

In order to guide model development, the following questions were asked: (1) what is the distribution of Dall's porpoise in the California Current Ecosystem? (2) what are the habitat preferences of Dall's porpoise in the California Current Ecosystem with respect to oceanographic variables? (3) is Dall's porpoise distribution better predicted by dynamic (oceanographic) or static (bathymetric) variables? (4) do distribution patterns predicted through acoustic and visual models differ?

Methods

Field Methods

The current distribution and habitat requirements of Dall's porpoise off the US West coast were determined using acoustic detections and visual sightings conducted on the NOAA vessel *McArthur* II from July 28- November 30, 2008 on the ORCAWALE cruise using systematic ship-based line-transect methods. The survey area encompassed waters off the US West coast out to 300nmi and was chosen to cover all waters within the California current ecosystem accessible to U.S. research vessels. The cruise ran on a transect line grid pattern with distances of 60nmi between each East-West line and at a speed of 10 knots (Fig 5.1).

At-sea data collection included geographical position, ship heading and speed, viewing conditions, sea-surface temperature (SST), Beaufort sea state, salinity and chlorophyll fluorescence. Sea-surface salinity and temperature were collected

continuously using a thermosalinograph sensor mounted at a depth of 3 meters. Expendable bathythermographs were deployed five times a day to measure the mixed layer depth (MLD). In addition, conductivity-temperature-depth (CTD) casts were conducted every evening that also measured MLD. CTD surface samples along with bucket samples taken 3-5 times per day collected data for measurement of surface chlorophyll. In addition to these habitat variables collected in the field, bathymetric variables were collected from ETOPO2 2-minute global relief data. Water depth, slope, aspect and distance from the 2000-m isobath were extracted from bathymetric data using ArcGIS tools (version 10.1, ESRI, Inc.).

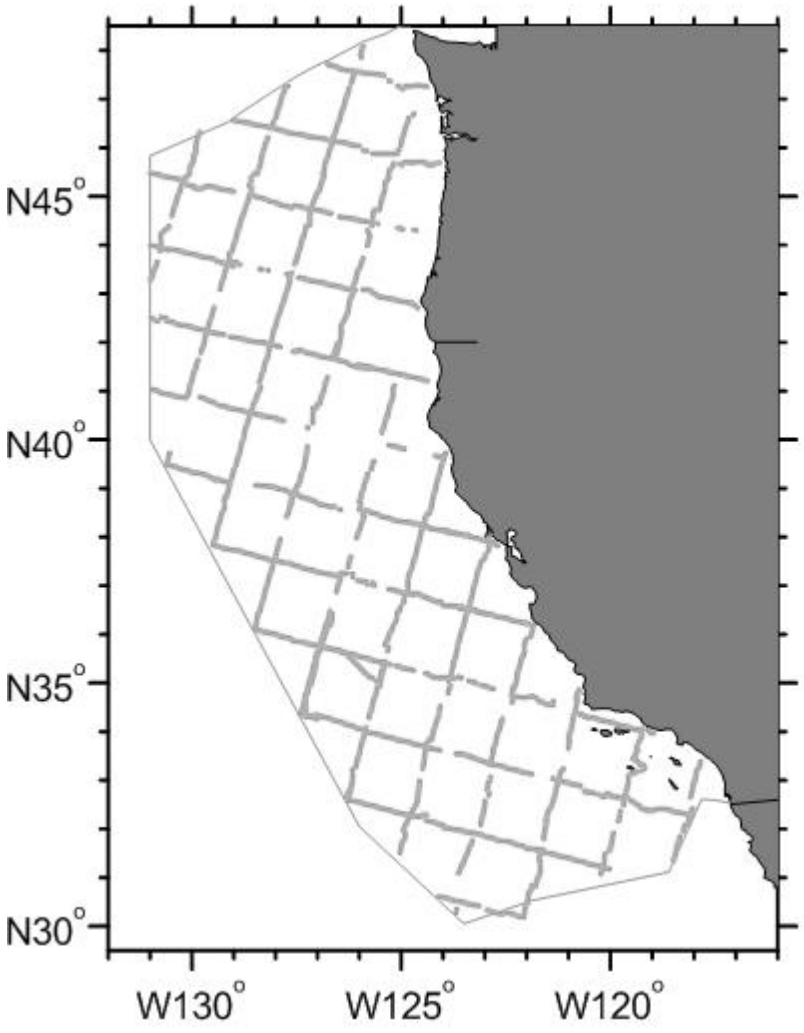

Fig. 5.1: Completed transects for the 2008 ORCAWALE cruise.

Dedicated marine mammal observers collected cetacean sighting data from the ship's flying bridge along all tracklines. Observers rotated between 3 stations with the left and right observers using 25 x 150 mounted binoculars and the central observer using hand-held binoculars or searching with the naked eye. Upon any marine mammal sightings, time, position, distance and bearing from ship, species identification, group composition and group size was recorded. If the marine mammal was within ~ 5.5km of

the trackline, the ship would generally divert from the trackline in order to estimate group size and species composition. Any sightings initiated during these diversions from the trackline were not included in the models since they were "off effort" sightings. For Dall's porpoise, species and group sizes could often be determined from the transect line, so off-effort diversions were rare for sightings of this species.

A five-element hydrophone array was towed approximately 300m from the stern of the boat at a depth of 4-8m during daylight hours to detect echolocation clicks. The array consisted of two mid-frequency hydrophones (EDO ceramic with a frequency response of 500Hz to 55 kHz ±5 dB and sensitivity of -155 dB re 1V/µPa after 40 dB pre-amplification) and three high-frequency hydrophones (Reson TC4013 hydrophones with a frequency response of 1.5 to 150 kHz ±3 dB with a sensitivity of -170 dB re 1V/µPa after 40 dB pre-amplification). Rainbow Click software was used to automatically detect clicks made by Dall's porpoise using data recorded from the high-frequency hydrophones. The program distinguished the clicks from other species in real-time by comparing frequency bands. IFAW's Logger 2000 software was used with Rainbow Click to record GPS locations and plot detected porpoise clicks on a real-time spectrographic display which was monitored continuously. Data were digitized and saved in 5-minute files to be post-processed for confirmation of Dall's porpoise detections. A total of 762 hours of recordings were made during 11,465km of survey trackline.

Analytical Methods

Click files were reviewed in Rainbow Click using five criteria including (1) the number of clicks, (2) localization of clicks, (3) wave form, (4) power spectrum and peak frequency and (5) time-frequency structure as viewed through a Wigner-Ville

transformation plot. Each detection was then categorized as either a “definite”, “probable” or “possible” Dall’s porpoise depending on the degree to which each click met all of the above criteria. Detections with 5 or more clicks, clear localization patterns (ie. some clicks were not along the beam), a clean wave form, a peak frequency between 120 and 137kHz and a Wigner plot with a strong single energy peak were classified as “definite”. Detections meeting these criteria but with only three or four clicks in a series were labeled as “probable”. If the detection had only two clicks in a series, but all other characteristics were shared with the “probable” assessment, then it was categorized as “possible”.

Porpoise sighting and acoustic detection data were then divided into 5km segments following Becker et al. (2010). Since all sections of continuous survey effort could not be evenly divided into 5-km segments, leftover segments were treated according to their length. If the segment distance was <2.5km, it was added randomly to one of the 5km segments within that continuous section of “on effort” trackline. If the segment was >2.5km, a new randomly placed segment was created within that continuous section of survey effort. The resulting segmented transect data sets included 2,361 segments for acoustic effort and 2,556 segments for visual effort. The “on effort” segments vary between these two methods since the visual observers are off effort during conditions above Beaufort 5 because sightings are limited during rough weather. Acoustic effort can continue during Beaufort conditions of 0-6 but if visuals diverged from the trackline in order to confirm a species or group size, acoustics effort was no longer considered standardized and any detections made during these periods were not considered in the models. Sightings and detections were each associated with segment

midpoints. Habitat data were also associated with segment midpoints and then interpolated to create continuous spatial grids between transect lines. Latitude and longitude were not included since they are static variables that do not reflect habitat as well as the dynamic oceanographic variables listed above. The resulting two databases of visual and acoustic effort, sightings/detections and habitat data were then used to construct two separate sets of habitat models.

Encounter rates of Dall's porpoise were predicted using a generalized additive model (GAM)- framework that related Dall's porpoise acoustic and visual encounters per km (the response variable) to the various oceanographic measurements mentioned above (explanatory variables). GAMs are nonparametric models that can accommodate many different types of relationships between the examined variables and are therefore particularly effective at modeling complex ecological relationships. A GAM may be represented as

$$g(\mu) = \alpha + \sum_{j=1}^{p} f_j(X_j)$$

(Hastie and Tibshirani 1990).

$g(\mu)$ is the link function, which relates the mean of the response variable, given the predictor variables $\mu = E(Y|X1,\ldots,Xp)$, to the additive predictor $\alpha + \Sigma jfj(Xj)$. We used a quasi-poisson link function with numbers of detections per segment as the dependent variable and the natural logarithm of segment length as an offset to account for differing segment lengths.

Encounter rate models were built separately for visual and acoustic survey data using forward-backward step-wise model building in S-PLUS (version 8.2) beginning

with a null hypothesis that Dall's porpoise have a uniform distribution with respect to all habitat variables. Habitat variables were sequentially added into the model and the significance of each explanatory variable was assessed with Akaike's Information Criterion (AIC) (Akaike, 1973). Two sets of models were built, one that included Beaufort sea-state as a variable and one that did not. While we would not predict that acoustic detections of Dall's porpoise wouldbe impacted by Beaufort sea-state, it may be a proxy for other characteristics of the local habitat. Since sightings are limited in rough weather, visual models were built with data collected in Beaufort states zero through five while acoustic models included data collected in Beaufort zero through six. The model with the best fit using the fewest number of explanatory variables was selected. Models were evaluated by both statistical and qualitative methods. Spatial ratios of sightings or acoustic detections to model-predicted encounter rates (observed/predicted) were generated to evaluate the predictive capabilities of each model. In addition, the percentage of explained deviance was compared across models. Lastly, the best encounter rate model for both the visual and acoustic datasets was then used to predict encounter rates across a 5km x 5km grid of the entire study area. These grid predictions were interpolated to produce smoothed average encounter rates, andthese smoothed encounter rates were then mapped. Sightings and acoustic detections were plotted on these maps to allow for visual comparison of the geographic predictions and the *in-situ* species detections.

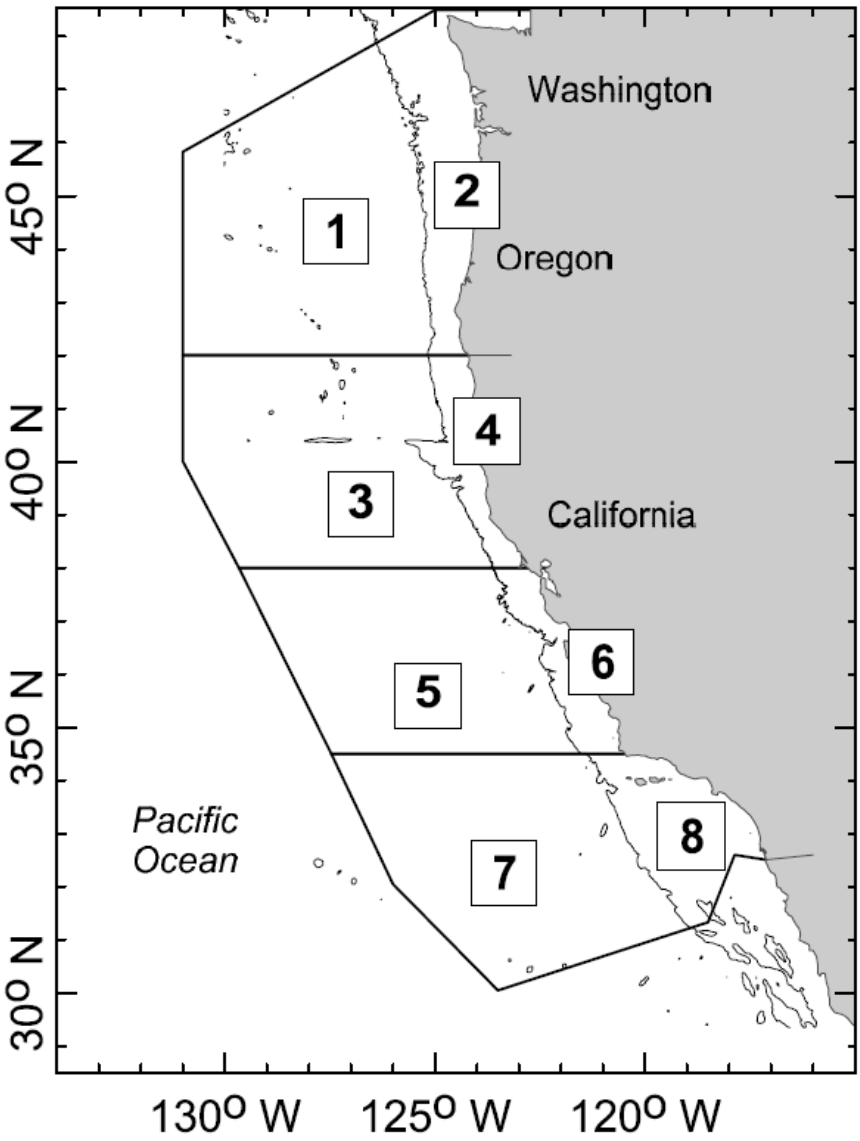

Fig. 5.2: Geographic regions used for evaluation of spatial predictions of encounter rates. The north-south line through the study area represents the 2000m isobath.

Results

During the cruise, there were 79 sightings of Dall's porpoise which were subsequently assigned to 71 segments (Table 5.1). Post-processing of acoustic data resulted in 118 detections of Dall's porpoise; 45 of these were classified as definite, 31 were probable and 42 were possible. However, many of these detections were made

while acoustics was “off effort”. The number of detections that were made while acoustics was “on effort” and therefore assigned to a segment and used in model building was 44 total with 28 definite, 10 probable and 6 possible detections (Tables 5.1 & 5.2). 14 acoustic detections of Dall’s porpoise were also sighted by the visual team simultaneously (Table 5.1). Both visual and acoustic detections of Dall’s porpoise were more common inshore and north of 38°N (Table 5.2).

Table 5.1: Summary of total visual, acoustic and dual detections of Dall’s porpoise from the 2008 ORCAWALE survey.

	# of events
Visual sightings	79
Acoustic detections	44
Detected by both methods	14

Table 5.2: Spatial summary of acoustic and visual detections of Dall’s porpoise from the 2008 ORCAWALE survey.

	All acoustic	Probable & Definite acoustic	Definite acoustic	Visual
WA/OR inshore	8	8	8	24
WA/OR offshore	2	2	2	3
NorCal inshore	16	14	11	34
NorCal offshore	1	1	1	2
CenCal inshore	12	9	4	10
CenCal offshore	1	1	1	6
SoCal Inshore	4	3	1	0
SoCal offshore	0	0	0	0
Total	44	38	28	79

Correlations between oceanographic measurements were reviewed. Correlations were all below 0.68 except for SST and chlorophyll which was -0.84. Overall, as distance

from shore and depth increased, mixed layer depth increased, chlorophyll decreased, salinity increased and water temperatures were generally warmer.

Selected Habitat Predictor Variables: Visual Models

Including Beaufort Sea-state

The final best model for Dall's porpoise encounter rates built with visual data included depth, slope, aspect, SST, SSS, Beaufort condition and distance from the 2000-m isobath (Fig. 5.3(a)). While nearly all habitat variables showed an inverse relationship to Dall's porpoise encounter rates, sightings declined most dramatically with increasing distance from the 2000m isobath, slope, SST and Beaufort sea-state (Fig. 5.3(a)). Dall's porpoise were most commonly found in waters with SST between 12 and 14°C. A nearly linear decline in sightings resulted with increasing Beaufort state since the species is difficult to detect in rough seas (Fig. 5.3(a)).

Without Beaufort Sea-state

The model that did not include Beaufort sea-state selected fewer predictor variables, including slope, aspect, SST, SSS and distance from the 2000m isobaths (Fig. 5.3(b)). The functional form of the relationship between Dall's porpoise and each of the variables was largely similar to those in the model with Beaufort (Fig. 5.3(b)).

Selected Habitat Predictor Variables: Acoustic Models

Three sets of models were built with acoustic data, one using the definite detections (Figs. 5.3(c) & 5.3(d)), one with probable and definite detections (Fig. 5.3(e)) and one with all detections (Fig.5.8). The only model in which Beaufort state was selected as a predictor variable was in the model built exclusively with definite detections (Fig. 5.3(d)). In contrast to the visual model, the acoustic encounter rate of Dall's porpoise increased with increasing Beaufort state (Fig. 5.3(d)). This "definite" detections

model also included slope, SST and distance to the 2000m isobath. Encounter rate decreased with increasing slope and distance from the 2000m isobaths (Fig. 5.3(c) & 5.3(d)). For the model built with probable and definite detections, only SST and MLD were selected (Fig. 5.3(e)). Detections of Dall's were most common in waters with a SST of 12-13°C and with a MLD of 20-35m (Fig. 5.3(e)). The best model for all acoustic detections of Dall's porpoise included slope, SST and MLD (Fig. 5.3(f)). Model functional forms between Dall's porpoise encounter rates and slope, SST and MLD in the "all" detections model were the same as those observed in the other acoustic models.

Metrics of Model Performance

Spatially-explicit investigations of model predictive performance were carried out by examining ratios of observations to predicted encounter rates (Table 5.3). Models with high predictive performance will show the best fit to the data and have a spatial ratio close to 1. These ratios allow for investigation of the regional differences in model performance. Ratios of observed to predicted encounter rates averaged across the entire study area were very similar across all models. However, regionally-specific ratios varied slightly. In general, all models performed better in Northern California waters and off Washington and Oregon. Additionally, all models had greater predictive success in inshore waters than offshore areas. While the addition of Beaufort to the "definite" model improved performance in some regions, it decreased performance in others. In the visual model, performance increased slightly in the northern regions with the inclusion of Beaufort but decreased in southern regions. The acoustic model that included Beaufort did not demonstrate any inshore-offshore or north-south pattern in the influence of this variable on predictive performance.

Model performance was also assessed with measures of deviance (Table 5.4). Explained deviance was highest for the visual model with Beaufort included. The inclusion of Beaufort did not make a substantial change in explained deviance for the acoustic models. Acoustic models built with the definite detections fit the data better than those built with all detections or the probable and definite detections.

Encounter rate maps

Inspection of final encounter rate maps show that visual and acoustic methods result in slightly shifted predicted distributions of Dall's porpoise (Fig. 5.9). The visual predictions are focused around Cape Mendocino while the acoustic detections have a hot spot slightly south off Pt Reyes. The acoustic models built with definite, probable and all acoustic detections also differ slightly. The encounter rate map made from probable and definite detections has a smoother predicted distribution than the map created with the definite detections only.

Table 5.3: Spatial ratios of observations or detections to model predicted encounter rates (observed/predicted) for all models.

Region	All acoustic	Prob/Def acoustic	Definite acoustic	Def. acoustic w/Beaufort	Visual	Visual w/Beaufort
WA/OR inshore	1.12863	1.338679	1.419045	1.287204	0.928596	1.031787
WA/OR offshore	1.185852	1.153149	0.781986	0.729604	0.332846	0.487146
NorCal inshore	1.060427	0.995106	1.179508	1.184929	1.379786	1.107742
NorCal offshore	0.643588	0.527128	0.474829	0.576251	0.522238	0.480618
CenCal inshore	1.06988	1.079831	0.820527	0.91852	1.161438	1.029394
CenCal offshore	0.502049	0.456182	0.734438	0.619565	1.834579	2.993448
SoCal Inshore	0.809934	0.887055	0.509778	0.53921	0	0
SoCal offshore	0	0	0	0	0	0
Entire Survey Area	0.999934	0.99998	0.999871	0.999866	0.999952	0.999902

Table 5.4: Dispersion, deviance and explained deviance model valuation statistics for all models.

	Dispersion	Null	Deviance	Explained Deviance
All acoustic	1.2904534	350.6385	288.2257	0.1779977
Prob/Def acoustic	0.856648	313.8594	252.8625	0.1943447
Definite acoustic	0.4008058	249.0251	179.233	0.2802614
Def. acoustic w/Beaufort	0.3763966	249.0251	172.0556	0.3090833
Visual	0.906426768	567.8053	405.5348	0.285785459
Visual w/Beaufort	0.763839767	567.8053	356.9159	0.37

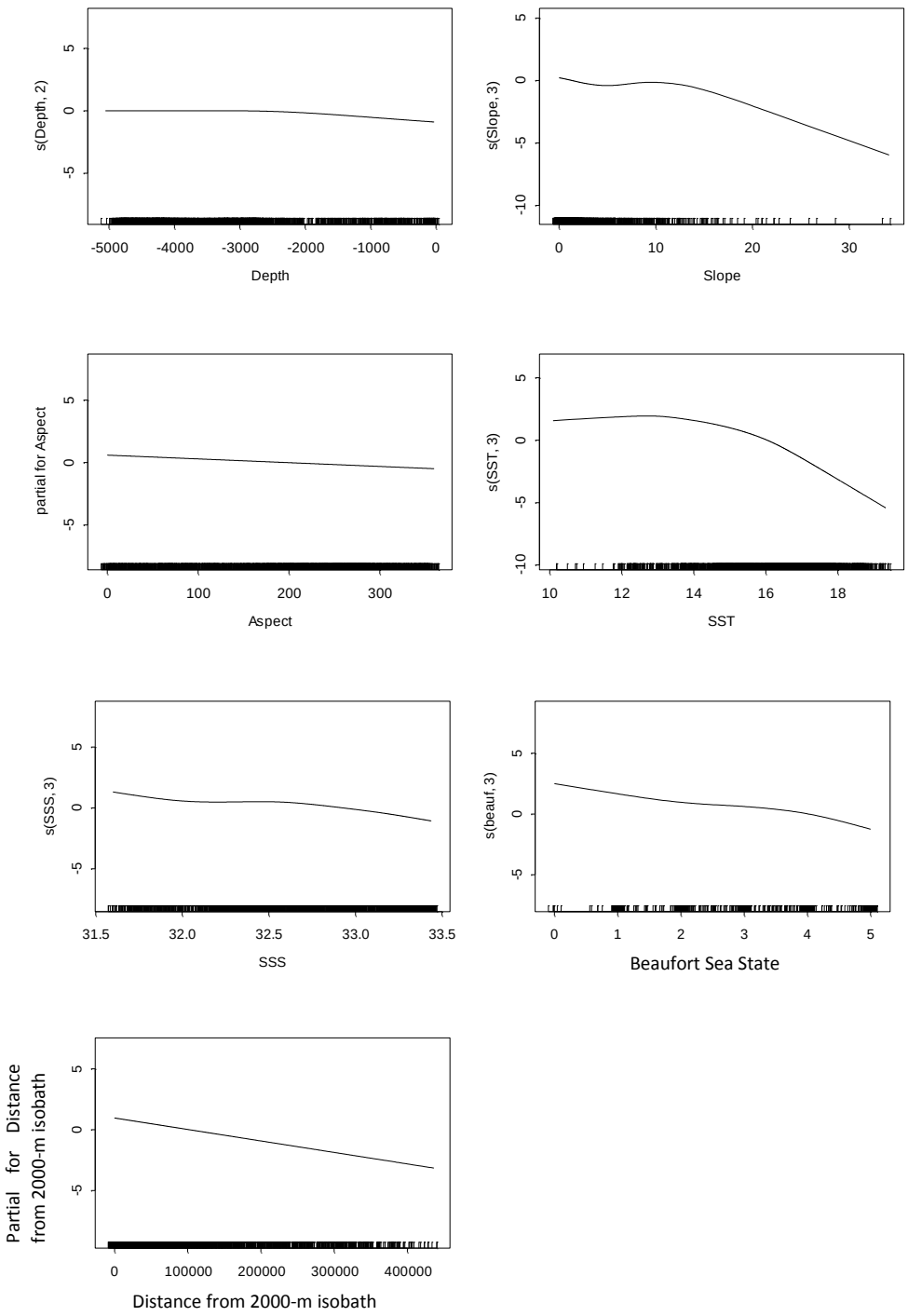

Fig. 5.3(a): Scaled encounter rate model functions for visually detected Dall's porpoise with Beaufort sea-state included. Models were built with both linear terms and smoothing splines ('s' on y-axis) with up to 3 degrees of freedom. Degrees of freedom for nonlinear fits are in parentheses on y-axis. The y-axes represent the term's function (linear or spline). Zero on y-axes indicate no effect of the predictor variable on Dall's porpoise encounter rate. Y-axes have been scaled to show relative effects of predictor variables on encounter rate. Predictor variables include sea surface temperature (SST), slope, depth, aspect, sea surface salinity (SSS), Beaufort sea state (beauf), and distance from the 2000-m isobath.

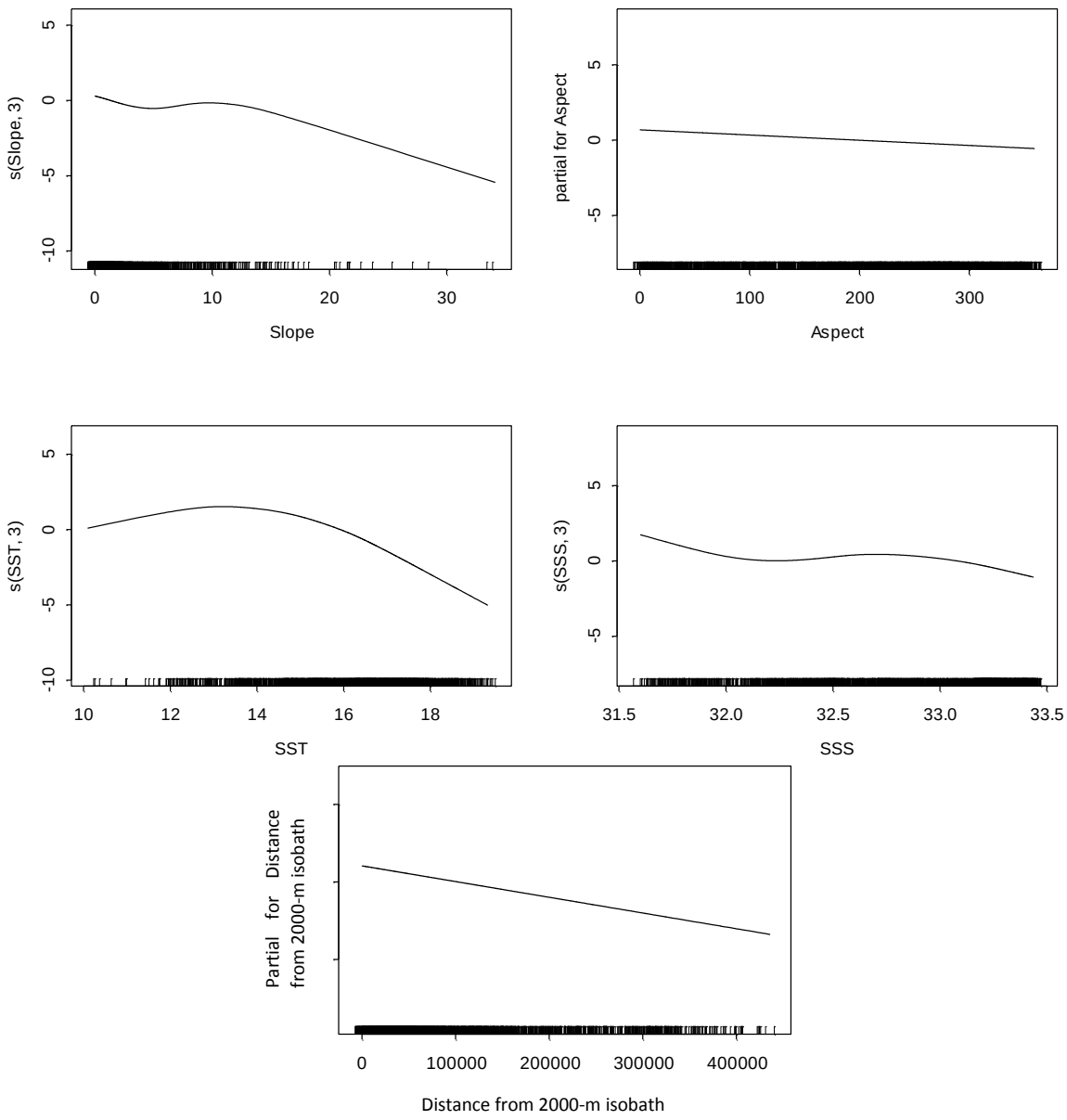

Fig. 5.3 cont. (b): Scaled encounter rate model functions for visually sighted Dall's porpoise models without Beaufort sea-state.

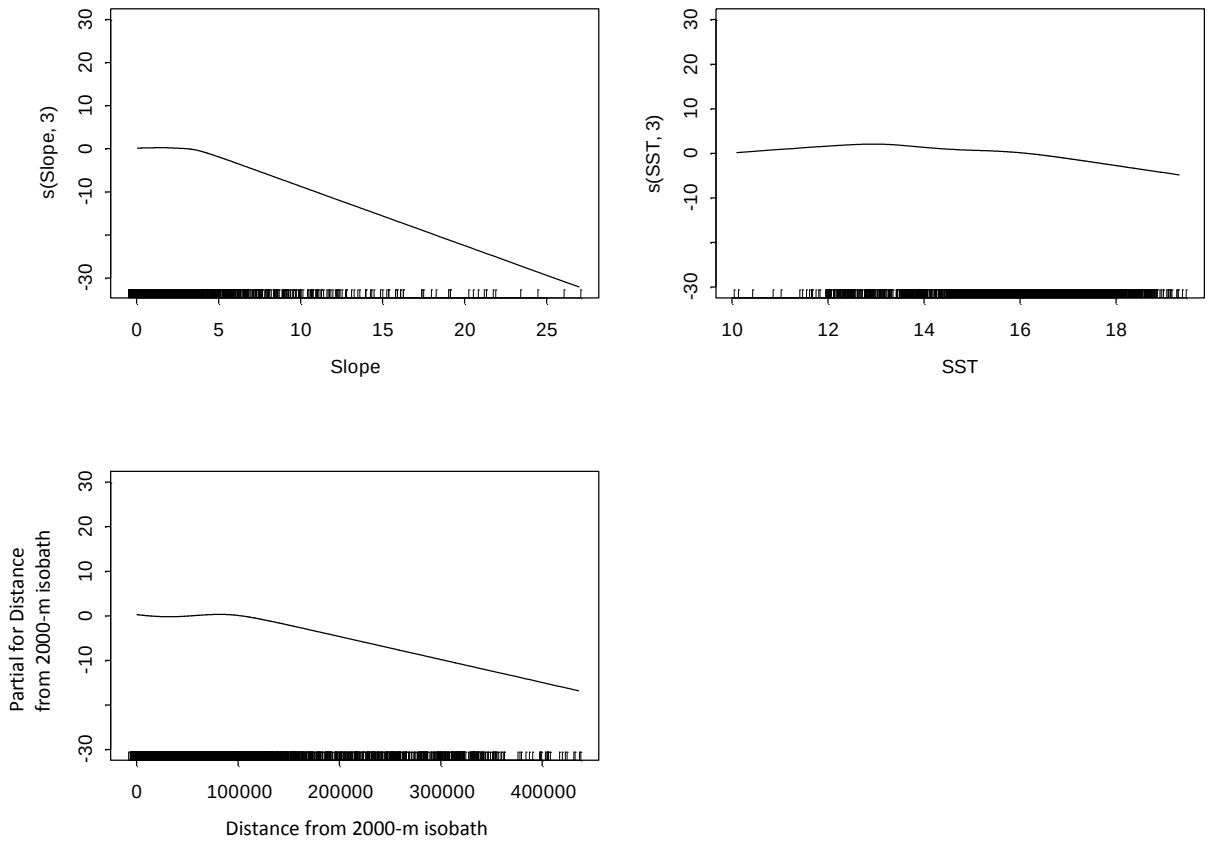

Fig. 5.3 cont. (c): Scaled encounter rate model functions for acoustically detected Dall's porpoise models built with "definite" detections without Beaufort sea-state.

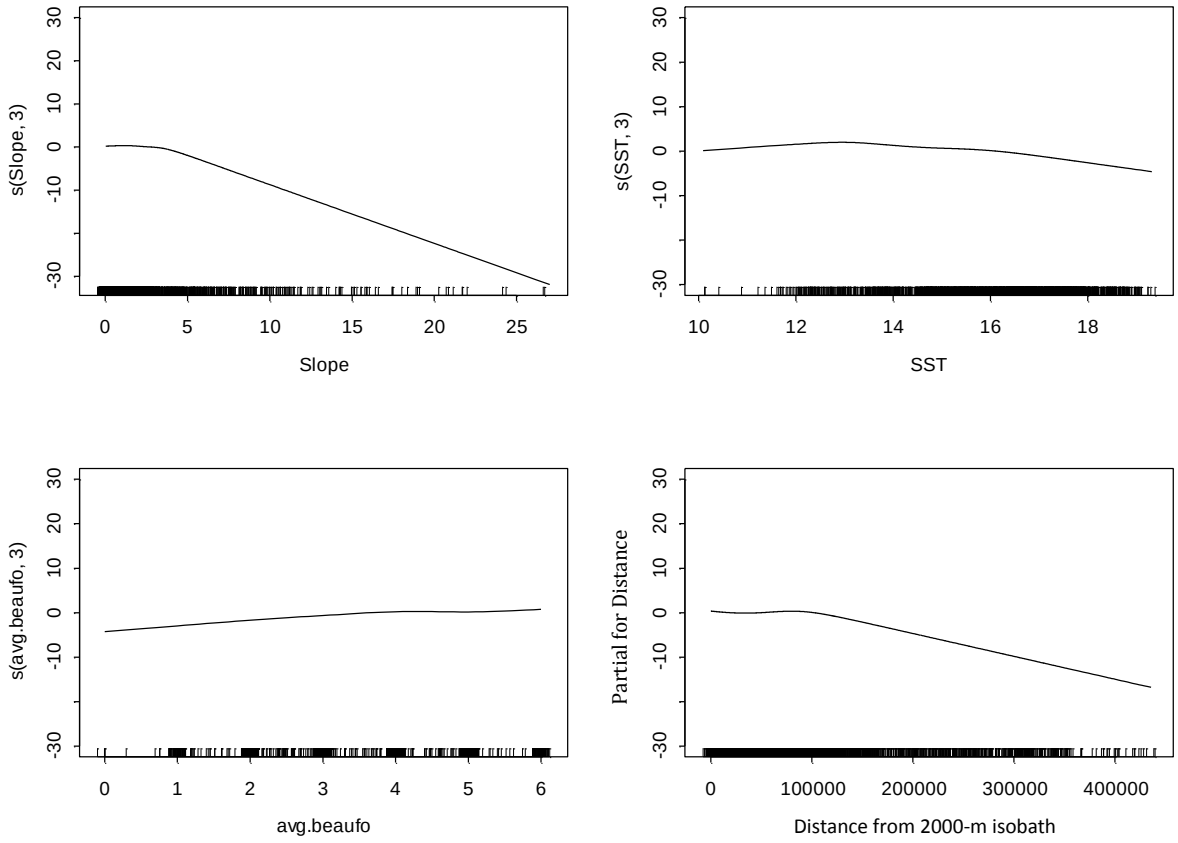

Fig. 5.3 cont. (d): Scaled encounter rate model functions for acoustically detected Dall's porpoise models built with "definite" detections with Beaufort sea-state included.

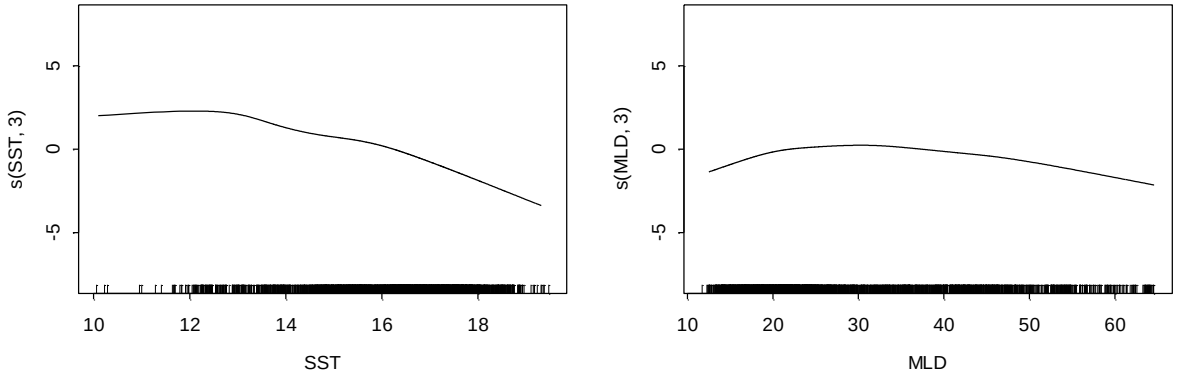

Fig. 5.3 cont. (e): Scaled encounter rate model functions for acoustically detected Dall's porpoise models built with "probable and definite" detections without Beaufort sea-state.

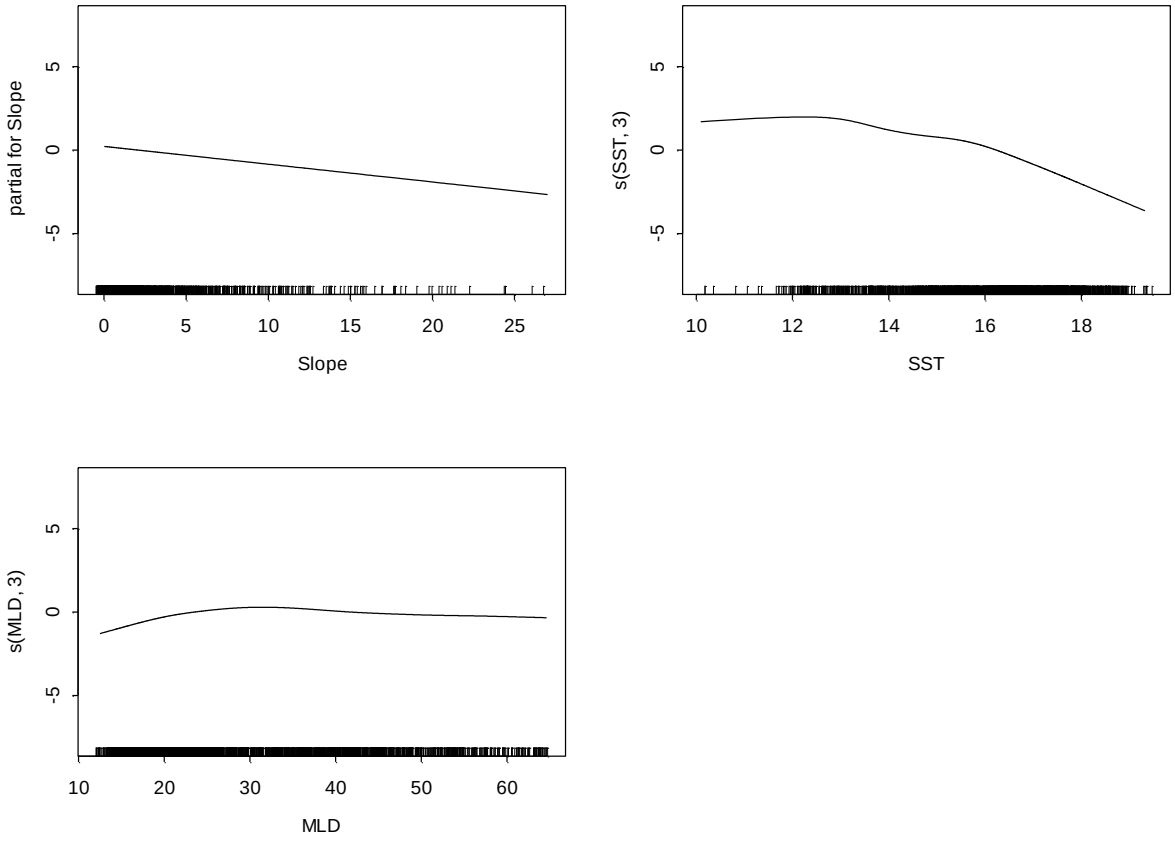

Fig 5.3 cont. (f): Scaled encounter rate model functions for acoustically detected Dall's porpoise models built with "all" detections without Beaufort sea-state.

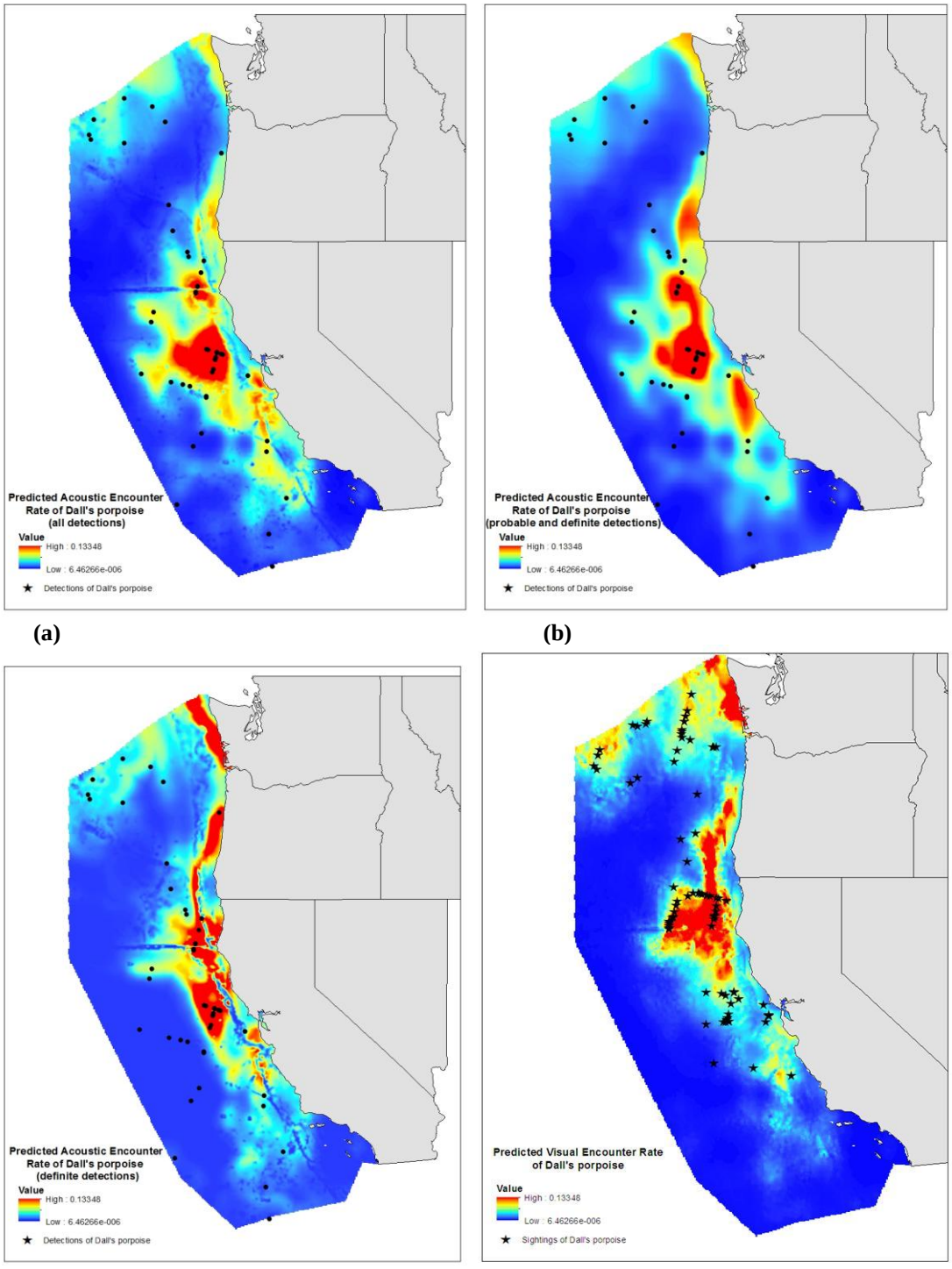

Fig 5.4: Modeled encounter rates for Dall's porpoise in the CCE in 2008. Models are (a) "all acoustic detections, (b) "probable and definite" acoustic detections, (c) "definite" acoustic detections and (d) visual sightings. Black dots in panels (a) to (c) are acoustic detections and black stars in panel (d) are sighting locations.

Discussion

In 2008, the distribution of Dall's porpoise was centered off northern California in cool waters, close to the 2000m isobath. There was general agreement between models built with acoustic data and those with visual data in predicting Dall's porpoise encounter rate distribution patterns (Fig. 5.4). However, the distributions were slightly shifted latitudinally with the highest probability of encounters predicted by acoustics slightly south of that predicted by visual methods. If both the visual and acoustic encounter rate maps are viewed together, the additive distribution of the two is similar to that predicted by previously published average models built with multiple years of data over a 20-year time frame (Fig. 5.5), confirming that results of our single year models general agree with what is already known about Dall's porpoise distribution (Keiper *et al.*, 2005; Barlow & Forney, 2007; Barlow *et al.*, 2009). These long-term models provide a more synoptic view of the species distribution over variable habitat conditions. By combining both acoustic and visual methods in a single survey year, our sample size increased and detections could be made under a variety of conditions across the entire CCE. This combination provided a more thorough assessment of Dall's porpoise distribution. Differences that exist between our model for 2008 and the average long-term models highlight the high degree of spatial and temporal variability in the CCE. Dall's porpoise clearly respond to this variability and the distribution of the population within a single year may shift annually.

Most of the models included both static and dynamic variables as important predictor variables, suggesting that both bathymetric and biological factors influence Dall's porpoise encounter rates. SST was the only variable shared across all of the visual

and acoustic-based models. Dall's porpoise were most commonly found in 12-14°C waters but acoustic detections also showed Dall's porpoise to occasionally be present in slightly cooler temperature water as well, around 10-11°C. This may reflect the ability of acoustics to detect animals in rougher seas which are often found in the more northern regions of our study area where waters are cooler. Slope was the second most commonly selected variable by the models. However, there was discrepancy between the visual and acoustic models in the slope values where Dall's porpoise was found. Visual-based models showed that Dall's porpoise was most commonly encountered over slopes of 0-10° while acoustic detections of the species dropped off steeply after 5°. This again may be related to geographical variability in detection abilities between the two methods. Alternatively, it may be reflecting behavioral state variability in detection abilities between acoustic and visual methods. Though little is known about Dall's porpoise acoustic behavior, echolocation clicks are typically produced for locating prey (Kyhn *et al.* 2013). Dall's porpoise are known to feed on both epipelagic and mesopelagic fish and cephalopods (Fiscus *et al.*, 1980; Okamoto *et al.*, 2010; Ohizumi *et al.*, 2003). If acoustics is mostly detecting foraging animals, many of these individuals may be at depth at the time of detection and therefore unavailable to visual sightings. Therefore, it is possible that acoustic detections and visual detections are effectively sampling different behavioral states. If that is the case, the observed difference in slope angle between acoustic and visual detections may be related to foraging and non-foraging habitats.

This reasoning may also inform interpretation of the inclusion of MLD in two of the acoustic models and its absence from the visual models. MLD is also likely related to foraging and may reflect geographic differences in behavior and detection of Dall's

porpoise. MLD is typically shallower in warmer, more stratified and less nutrient-rich waters and deeper in areas of higher upwelling and related productivity (Gargett, 1997). Dall's porpoise was found in areas with mixed layer depths of 20-40 meters. It has been shown that there exists an "optimum stability window" of water column stability for the productivity and survival of various species of zooplankton and fish in the CCE (Gargett, 1997). The relationship between Dall's porpoise acoustic detections and MLD may therefore be a function of Dall's porpoise prey distributions in response to favorable oceanographic conditions.

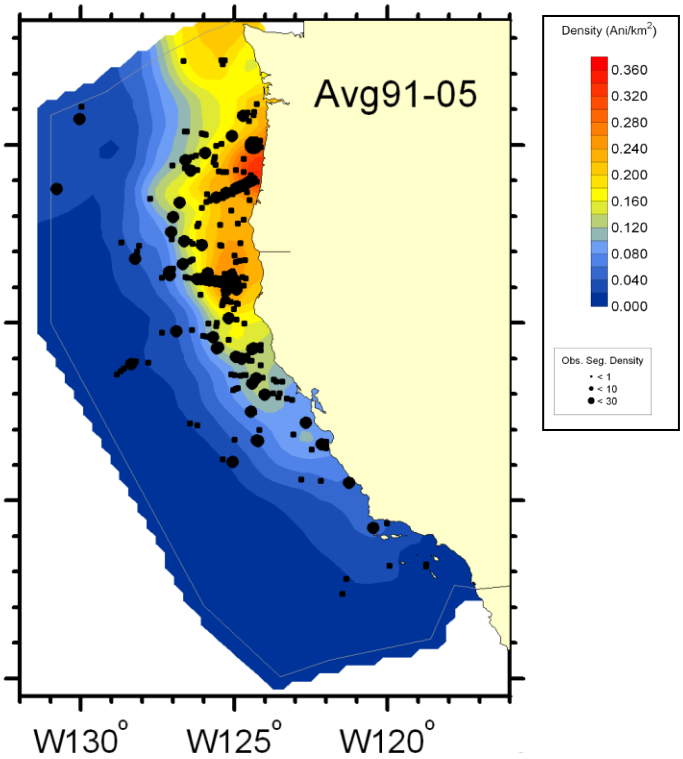

Fig. 5.5: Dall's porpoise habitat model created from visual data from 1991-2005 (Barlow *et al.*, 2009). Black dots are sightings while color gradient indicates predicted porpoise density.

One of the challenges in interpreting these models is the dearth of fundamental behavioral, acoustic and biological information available for Dall's porpoise. While

there are records of Dall's porpoise stomach contents, foraging behavior is relatively poorly described and it remains unclear if the species feeds primarily at depth or on epipelagic species (Jefferson, 1988; Amano *et al.*, 1998). Additionally, it has been hypothesized that Dall's porpoise may feed primarily at night if they do feed mainly on mesopelagic species (Amano *et al.*, 1998). Many of the species that have been recorded as observed prey items typically decrease their depth in the water column at night (Fiscus *et al*, 1980). Physiological and anatomical evidence suggests that Dall's porpoise are relatively deep diving species and therefore would be able to utilize this resource (Ridgway & Johnston, 1966). If the species does primarily feed at night, it is also likely they would be more acoustically active at night. However, very little information exists on the frequency of Dall's porpoise vocalizations or the behavioral context for their vocalizations. Though they are believed to be frequent echolocators, during this cruise there were many visual sightings that were not acoustically detected. This suggests that the species is not always vocal, at least during daylight hours when our observations were taking place. If the species is more vocally active during the night, acoustic surveys during daylight hours may significantly underestimate species presence. Data on behavioral context for vocalizations would allow for greater ecological understanding of habitat use.

Dall's porpoise is an excellent species to study acoustically because few other species in the study area produce such high-frequency sounds. Harbor porpoise are found in the study area and produce very similar echo-location clicks (Barlow, 1995; Kastelein *et al.*, 2002), but their distribution is largely limited to shelf waters less than 100m depth (Carretta *et al.*, 2001). Towed hydrophone data were not collected in shallow waters

during the 2008 survey to avoid entanglement in crab pots. Pygmy and dwarf sperm whales (*Kogia breviceps* and *K. sima*) are also found in the study area there and produce echolocation clicks with frequencies higher than 100 kHz (Madsen *et al.*, 2005). Descriptions of their signals are currently inadequate to know whether the criteria used here distinguishes them from Dall's porpoise. *Kogia* spp. are found broadly in deep waters worldwide, and if some *Kogia* detections are included in our acoustic data, the habitat characteristics of Dall's porpoise might be obscured. Better characterization of Kogia echolocation signals may improve the ability to discriminate between them and Dall's porpoise or validate our classification criteria.

Though details of habitat use patterns may be unavailable, the current methods and resulting models do allow for interpretations of relative habitat quality across the study area. The addition of acoustic detections increased the sample size of detections substantially. Acoustics allowed for detections under a greater range of conditions, through a more diverse suite of habitats and of potentially different behavioral states of the species. By examining the relationships between Dall's porpoise, detected both acoustically and visually, and physical and biological habitat variables, a dynamic and predictive understanding of habitat preference is achieved. It is clear from comparisons of the models presented here with models previously published of Dall's porpoise distribution in other single years in the CCE that the population's distribution shifts considerably from year to year (Barlow *et al*., 2009; Becker *et al*., 2012). The ability to more thoroughly capture the true distribution in any given year provides a better understanding of the ecological relationships that drive population distribution in future years under different oceanographic conditions. This predictive capability enables

population abundance estimates to be refined. With this information, it may be possible to estimate what proportion of the population is in our study area during a survey and therefore eventually allow for the calculation of trends in abundance which are currently unavailable for this population. We recommend that future habitat modeling studies of cetaceans include passive acoustics as a second line of evidence of species presence. As this study has confirmed, capturing a greater number of individuals, and therefore a greater portion of the population, can improve single year models and advance our understanding of species habitat preferences and population distribution.

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Chapter 5, in full, is currently being prepared for submission for publication of the material. Fleming, AH, Yack, T, Barlow, J. The dissertation author was the primary investigator and author of this paper.

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Chapter 6.

Conclusions

This dissertation has furthered understanding of temporospatial variability in cetacean population foraging ecology and habitat preferences in the California Current ecosystem. The cumulative findings of this work suggest that cetacean populations display a high degree of ecological adaptability. The focal ecosystem of this work is characterized by high variability in physical processes and biological responses. The populations of both species that were examined responded dynamically to the oceanographic and ecological change within this ecosystem over multiyear temporal scales and ecosystem-wide spatial scales. Through adjustments of their diet and distribution, the populations tracked variability in physical and biological ecosystem conditions, from climatic forces to prey densities. This emphasizes the integrative nature of cetaceans and their utility as indicator species. The greater resolution of cetacean ecological adaptability gained from this research also allows for better prediction of future population responses to climatic change. Additionally, this ecological adaptability highlights the need for long-term temporal and broad-scale spatial coverage of datasets to fully capture the ecology of these taxa and the potential processes that contribute to population structure at both demographic and evolutionary levels.

Chapter 2 demonstrated the difficulty in identifying population units below the species level for conservation designations. The National Marine Fisheries Service assessment of humpback whale endangerment was dependent on thorough information on population structure and connectivity. While abundances, threats and demographics were

also important to the assessment, absence of population structure data caused the greatest uncertainty in Endangered Species Act evaluation of this species. Complete description of the population structure of a globally distributed species takes decades, hundreds of researchers and thousands of samples. While this may be an unrealistic level of research effort for some populations or species, diversification of data types may aid in the description of population structure. By employing multiple lines of evidence of population identity (photographic identification, genetic data, stable isotope analysis and contaminant levels) structuring at both demographic and evolutionary scales may be better resolved.

Chapter 3 revealed that humpback whale feeding behavior is highly variable over multiyear time scales. The California Current Ecosystem population of humpback whales likely alters their primary prey source depending on local densities and availability of prey species. This shift in prey appears to be from krill to forage fish species, such as sardine and anchovy, and back again, representing a diet shift of approximately one trophic level. These changes in diet are correlated to larger scale ecological variability in the system including differences in temperature, upwelling and productivity between years. The degree of observed variability in CCE humpback whale diet significantly hinders interpretation of population structure based on stable isotope ratios. In order to use stable isotope ratios for geographic assignment, the magnitude of baseline variation within all populations across the habitat would need to be assessed.

Chapter 4 documented the scope of isotopic variability seen within individual humpback whales as a result of diet, physiological process, or tissue preservation method. Storage in DMSO was found to significantly impact $\delta^{13}C$ signatures but not $\delta^{15}N$

signatures. However, the magnitude of observed change was substantially less than the degree of change observed between years in the CCE population of humpback whales studied in Chapter 3, thereby supporting the interpretation of a switch in prey base as the most parsimonious explanation. Intrannual variability of stable isotope ratios within an individual was found to increase over time and reflect migratory behavior. The results of this study suggest that both preservative effects and individual variability in stable isotope signatures is not prohibitive for investigations of trophic differentiation in cetaceans if the trophic shift is larger than appromximately 1‰ in δ13C.

Chapter 5 explored the habitat preference of Dall's porpoise through a novel application of passive acoustics data to habitat modeling. Dall's porpoise distribution was found to be predicted by a combination of hydrodynamic features and static habitat variables. Population distribution changes over annual time scales in response to shifting oceanographic conditions with the offshore extent constrained by the species apparent preference for habitat over the continental shelf and slope. Acoustic detections expanded the diversity of environmental habitats and weather conditions that Dall's porpoise could be sighted in, improving sample size and resulting habitat models. Predictive understanding of habitat preferences enables better estimation of population range, distribution, abundance and structure.

The factors that lead to population divergence and speciation are complex. There exists a large body of literature on ecological speciation theory and a small, but growing, body of literature on empirical applications of ecological metrics to identification of demographically distinct populations (Schluter, 2001; Wolf *et al.*, 2008; Foote *et al.*, 2013). However, evidence from this dissertation research suggests that the cetacean

populations evaluated here have wide ecological niche breadth and adapt readily to changes in their surrounding environment. This adaptability would suggest that prey selectivity and habitat specialization, the characteristics examined here and often theorized to be catalysts for divergence, may be unlikely drivers of population differentiation in humpback whales and Dall's porpoise. For the observed populations, within population ecological variability included prey switches across trophic levels and habitat conditions with temperature ranges of 8°C. Both humpback whales and Dall's porpoise are known to be largely opportunistic foragers (Baker *et al*, 1985; Clapham et al., 1997; Geraci et al., 1989). Other species with narrower ecological niche breadths may be more suitable candidates for the application of ecological metrics to the identification of population structure. For species such as the blue whale (*Balaenoptera musculus)* that feed exclusively on krill, changes in stable isotope signatures would be more concrete evidence for geographic distinction between populations (Schoenherr, 1989). The efficacy of ecological characteristics as indicators of population structure may depend on the natural history, geographic range and foraging ecology of the taxon in question.

The observed ecological adaptability of humpback whale and Dall's porpoise populations also raises the question: what temporal and spatial scales are best suited for examining ecological divergence? A recent study examining ecological divergence of killer whales in the North Atlantic over milliennial time scales found little evidence of genetic isolation between two ecologically distinct groups that are thought to have had predominantly non-overlapping prey preferences for ~10,000 years (Foote *et al.,* 2013). However, numerous other studies on killer whales from different regions of the world have found significant genetic differentiation that likely resulted from feeding

specialization (LeDuc *et al.*, 2008; Morin *et al.*, 2010; Foote *et al.*, 2011). It seems plausible that different processes within one species may govern the outcomes of ecological divergence. With regards to spatial scales, a study of Galapagos sea lions found ecological, morphological and genetic divergence between two sympatric populations. The two sea lion populations occupy an area smaller than the geographic range of daily foraging trips of individuals and the authors concluded that ecological niche segregation was the most probable cause of differentiation (Wolf *et al.*, 2008). Despite the difficulty of resolving the relative importance of ecological drivers on ongoing speciation, it is clear that trophic constraints and opportunities were major factors in the evolution of Cetacea suborders and genera over the last 45 million years (Lipps & Mitchell, 1976).

While temporal and spatial scales of ecological divergence may influence population structure, social structure and cultural inheritance may also interact with ecological drivers of differentiation in a species-specific manner (Wolf *et al.*, 2008). The discrepancy between the killer whales and the sea lions mentioned above may be best explained by differences in social structure and cultural inheritance. Though both taxa are social, sea lion cultural inheritance of habitat preference and trophic niche would promote maintenance of population differentiation while cultural inheritance of foraging behavior in the killer whale populations is hypothesized to be more plastic and may not be evolutionarily transmitted (Wolf *et al.*, 2008; Foote *et al.*, 2013). Of my study species, humpback whales demonstrate a high degree of maternally-directed site fidelity whereby calves follow their mothers to specific breeding and feeding grounds during the first year of life and subsequently return to those same locations every year. This is thought to be a

major source of differentiation between humpback whale populations (Baker *et al.*,1993; Palsboll *et al*., 1995).

If ecological divergence may or may not lead to eventual speciation, this raises the question: Do ecologically divergent populations need to have evolutionary potential to be worthy of conservation? From a management perspective the answer to this question depends on the legislative goals. For example, the MMPA mandates that marine mammal populations be maintained as functioning elements of their ecosystem, emphasizing ecological diversity as a conservation goal. From a scientific perspective, biodiversity is often considered critical to ecosystem health and function. Cetacean populations such as the ones examined in this study are hypothesized to have significant ecological roles in the CCE ecosystem and their decline or loss would likely have significant impacts on ecosystem function through the alteration of energy flow, carbon sequestration, substrate disturbance, deep-sea biodiversity, and trophic interactions (Butman *et al.*,1995; Oliver & Slattery, 1985; Springer *et al.*, 2003). Though it is difficult to determine whether ecological characteristics of cetacean populations today will evolve into evolutionary distinctions in the future, it is clear that exploring cetacean ecological adaptability across multiple temporal and spatial scales can provide insight on both the ecological and evolutionary processes that create current cetacean population structure.

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