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Using Stable Isotopes To Assess Population Structure And Feeding Ecology Of North Pacific Humpback Whales (megaptera Novaeangliae)

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USING STABLE ISOTOPES TO ASSESS POPULATION STRUCTURE AND FEEDING ECOLOGY OF NORTH PACIFIC HUMPBACK WHALES (*MEGAPTERA NOVAEANGLIAE*)

by

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ABSTRACT

The North Pacific humpback whale (*Megaptera novaeangliae*) is a wide-ranging baleen whale species with a complex life history and population structure. As seasonal migrants, humpback whales are known to inhabit cooler, high-latitude waters when foraging and low-latitudes for mating and calving. Beyond this general migratory pattern, a number of demographic characteristics including, abundance, distribution, seasonal occurrence, and prey preferences remain unknown or poorly described. A complete understanding of humpback whale ecology is therefore lacking. Many methods used to explore these aspects of cetacean ecology are either prohibitively expensive or limited in the scope of what can be learned from their use. Fortunately, in recent years, the analysis of stable isotope ratios of animal tissues has proved a valuable and relatively inexpensive technique for providing information on trophic position, diet, and feeding origins of migratory populations. This study employed techniques in stable isotope ecology to increase knowledge of the population structure, migration routes, and foraging ecology of North Pacific humpback whales.

Skin samples were collected from free-ranging humpback whales throughout all known feeding and breeding grounds and were analyzed for stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope ratios. The population structure of humpback whales was first explored through geographic differences in stable isotope ratios. Stable isotope ratios varied significantly with location of sample collection. Based on this analysis, foraging animals were separated into six feeding groups. Classification tree analysis was then used to determine which isotopic variables could be used to predict group membership. Probable migratory linkages were then described by applying results of classification trees to $\delta^{13}C$ and $\delta^{15}N$ of animals sampled on breeding grounds.

Strong migratory connections between the eastern-most foraging and breeding areas and the western-most areas were reflected in similarities of stable isotope ratios.

Foraging ecology was then examined through calculation and comparison of the relative trophic levels of the six feeding groups. Isotopic values suggest some feeding groups are piscivorous, while others feed on a more mixed diet. These results can be used to determine if differences in diet composition between groups result in differences in accrued nutritional benefits, negatively impacting reproductive success and survival relative to fish eating groups.

Finally, to gain insight into specific foraging habits, the diet of one group of humpback whales was modeled using an isotope mixing model. The δ^{13}C and δ^{15}N of Kodiak Island, Alaska humpback whales and several species of potential prey indicate that these animals likely rely heavily on euphausiids (*Thysanoessa spinifera*), Pacific sandlance (*Ammodytes hexapterus*), and capelin (*Mallotus villosus*).

This study represents the first application of stable isotope ecology to an entire population of marine mammals. Stable isotope analysis was successfully applied to describe and improve understanding of the demographics of North Pacific humpback whales.

This dissertation is dedicated to Lynda C. MacRill, who was a loving aunt, mother, daughter, and sister and who shared with me her gift of kindness and generosity and to my mother, Patty, who taught me that a fear of drowning should never keep you from jumping in.

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CHAPTER 1: INTRODUCTION

Natural selection acts on individual animals throughout the life cycle. Because selection occurs at virtually all stages of this life cycle, nearly every aspect of an animal's life history and ecology is subject to selective pressures. Fitness is the potential of a given genotype to survive and reproduce in the face of this selection. Fitness on an individual level cannot be explicitly measured, but relative fitness can be indirectly measured through reproductive success and survival.

A key factor in determining the reproductive success and survival of individuals is the habitat they occupy (Gunnarsson et al. 2005). Choice of habitat influences both the quantity and quality of resources available to individuals, including mating opportunities and access to forage. Rarely will a single habitat provide the maximum availability of all necessary resources. As a result, most taxa compromise by balancing their resource needs such that overall survival and reproductive opportunities are maximized. A number of behaviors have evolved to maintain this balance, including seasonal migration.

The evolution of migration has enabled many taxa, including birds, insects, and mammals, to exploit the resources in habitats that would not be suitable for year-round residence (Aidley 1981b, Webster et al. 2002). Migration is described as the regular seasonal movement of individuals from one location to another and back, and most often occurs between breeding and nonbreeding locations. While migration is a behavior that presumably improves fitness, it also imposes numerous ecological pressures and consequences on individuals and populations (Studds & Marra 2005). Complex patterns of migratory habitat use can result in carry over effects, such that the consequences of one habitat occupancy affect individual success at the other habitat (Marra et al. 1998,

Gill et al. 2001, Norris et al. 2004). As a result, understanding how disparate habitats are used and connected is critical to the effective management of migratory populations. Exploring migratory connections is essential to understanding the complete ecology of an animal, including conservation, behavior, population dynamics, and reproductive success (Webster et al. 2002).

Each of these aspects of animal ecology is strongly influenced by the physical condition of migrants when they arrive on their respective breeding grounds. Poor body condition has been implicated in declines in reproductive success, lower annual survival rates, changes in offspring sex ratio, and delays in migratory timing in such taxa as passerine birds and baleen whales (Perrins 1970, Price et al. 1988, Wiley & Clapham 1993, Møller 1994, Stolt & Fransson 1995, Lozano et al. 1996, Sandberg & Moore 1996). The body condition of migratory birds may be limited primarily by habitat quality and food abundance, and reduced prey intake specifically can lower annual survival rates (Strong & Sherry 2000, Gill et al. 2001, Johnson & Sherry 2001). Many migratory species do not feed while on their breeding grounds. These animals undergo long periods of fasting, exposing individuals to periods of nutritional stress and potential reductions in body condition (e.g. baleen whales, sea birds). For these taxa, food quality and intake need to be optimized on the feeding grounds in order to sustain migration and breeding behaviors and, for females, lactation and pregnancy (Lockyer 1981a, Craig et al. 2003, McWilliams et al. 2004). Thus, studies directed at determining location of feeding and foraging ecology may arguably be the most critical in determining survival and reproductive success for a fasting, migratory species.

Migratory baleen whales fast during migration and while on breeding grounds but are known to be consumers of a highly varied diet on their selected feeding grounds (Lockyer & Brown 1981, Gaskin 1982). As a result, the presence of foraging whales can significantly affect ecosystem dynamics (Laws 1985, Katona & Whitehead 1988, Kenney et al. 1997). Unfortunately, the abundance, distribution, seasonal occurrence, and prey preferences of most large whale species are relatively unknown. This gap in knowledge is particularly troubling for migratory animals because, as stated above, a complete understanding of their ecology depends on linking all geographic regions used by individuals for breeding, feeding, and migratory routes (Hobson 1999).

The North Pacific humpback whale (*Megaptera novaeangliae*) represents one species of migratory baleen whale for which such knowledge is needed. These whales undergo extensive seasonal migrations and periods of fasting, spending summer months foraging in productive high latitude waters before migrating to lower latitudes to breed and give birth. Humpback whales do little or no feeding while on their breeding grounds and can lose 1/3 to 1/2 of their body mass (Dawbin 1966, Lockyer 1976, Baraff et al. 1991, Laerm et al. 1997). During this period of fasting, humpback whales rely almost exclusively on their blubber stores, which have accumulated while foraging on the high latitude feeding grounds (Lockyer 1981a).

Humpback whales belong to the family Balaenopteridae and are found in all major ocean basins. Weighing approximately two tons and measuring four to five meters at birth, the humpback whale will grow to nearly 30 tons and 13 to 15 meters in length. Sexual maturity is reached at between four and 12 years and physical maturity at 10 years of age (Chittleborough 1965, Clapham & Mayo 1987, Clapham 1992, Straley et al. 1994,

Gabriele et al. 2001). The oldest documented humpback whale was 48 years old when it was harvested by commercial whalers, but humpback whales are thought to have life spans similar to those of humans (Chittleborough 1965). Female humpback whales give birth to a single calf every two years following a 12-month gestation period (Chittleborough 1958, Straley et al. 1994). Calves are born on wintering grounds and migrate to feeding areas with their mothers (Dawbin 1966, Baker et al. 1987, Clapham 1996). Weaning is typically initiated at 5-6 months of age at which time calves will begin to feed on prey (Clapham & Mayo 1990). Separation of mother and calf is usually complete by the end of the calf's first year and can occur on summer or winter grounds or during migration (Baker & Herman 1984, Glockner-Ferrari & Ferrari 1984, Baker et al. 1987, Clapham & Mayo 1987, Baraff & Weinrich 1993, Straley 1994, Steiger & Calambokidis 2000).

Commercial whaling significantly reduced the number of humpback whales in the North Pacific. The North Pacific population of humpback whales is estimated to have numbered between 15,000 and 20,000 individuals before the commercial exploitation of this species began in the early 1900's (Rice 1977). Prior to international protection from harvest in 1967, humpback whales in the North Pacific may have been reduced to as few as 1,000 animals (Perry et al. 1990). The most current estimate of abundance for the entire North Pacific lists 18,302 animals (Calambokidis et al. 2008).

As a result of their commercial exploitation, humpback whales were listed as an endangered species in 1973 under the United States Endangered Species Act and as a threatened species on the IUCN Red List. Though humpback whales are experiencing population growth in the North Pacific, they are exposed to a number of anthropogenic

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threats, including vessel strikes, exposure to pollutants, and fishing gear entanglements (Angliss & Outlaw 2008).

Presently three stocks of humpback whales are recognized within the North Pacific based on winter breeding location. These stocks have been defined for the purpose of management and are used to assess human-caused mortality and estimate population parameters such as growth rate and abundance (Table 1.1; Angliss & Outlaw 2008). The three stocks are defined as: the eastern North Pacific (ENP), the central North Pacific (CNP), and the western North Pacific (WNP) (Angliss & Outlaw 2008; Figure 1.1). The exact population structure and migration routes of these stocks are not well known but existing data suggest these three stocks are relatively discrete: whales from the ENP stock winter in coastal Central America and Mexico and migrate to an area between the coasts of California and southern British Columbia (Calambokidis et al. 1989, Steiger et al. 1991, Calambokidis et al. 1993); whales from the CNP stock winter in the Hawaiian Islands and migrate to the areas of northern British Columbia and Prince William Sound and west to Unimak Pass, Alaska (Baker et al. 1990, Perry et al. 1990, Calambokidis et al. 1997); and whales from the WNP stock winter in Japan and migrate to the Bering Sea and waters west of the Kodiak Island archipelago (Berzin & Rovnin 1966, Nishiwaki 1966, Darling 1991). No feeding destination has been assigned to whales known to winter near Mexico's offshore islands, although some of these animals have been sighted in the western Gulf of Alaska (Witteveen et al. 2004; Figure 1.1). Thus, stock designations are only approximations of what is likely a much more complex population structure.

Geographically separate aggregations (see Table 1.1) are found within feeding grounds of each stock and several aggregations may migrate to a single breeding location for mating and calving (Waite et al. 1999, Urbán R et al. 2000). A small degree of movement (1 to 2%) between stocks and aggregations may occur, but, for the most part, they are isolated from one another (Baker et al. 1986, Calambokidis et al. 1996, Waite et al. 1999, Mizroch et al. 2004). Segregation of feeding aggregations of humpback whales in both the North Pacific and the North Atlantic has been attributed to a "cultural" transmission of fidelity to migratory destinations as a result of a calf's early maternal experience (Aidley 1981a, Martin et al. 1984, Baker et al. 1987, Clapham & Mayo 1987).

While on their feeding grounds, humpback whales are classified as apex predators and are known to feed on a highly varied diet, including euphausiids and small, schooling fish (Nemoto 1957, 1959, Krieger & Wing 1984, 1986). Many humpback whale prey species, such as Pacific herring (*Clupea pallasii*), are also targeted by other consumers including other marine mammals and commercial fisheries; Others, including foraging fish, are linked indirectly through complex food webs. If these prey resources are limited, such overlap may cause competition that could lead to reductions in the growth, reproduction, and survival of the predator populations.

The complex life history of the North Pacific humpback whale highlights the need for research in all areas of their ecology. As a migratory and endangered species, understanding the linkages between the different geographic areas used by individual humpback whales is critical in assessing effective conservation and recovery efforts. As a result of their fasting behavior, the quality of prey and its ability to contribute to energy reserves is critical to survival and reproductive success of humpback whales. Further, as

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an apex predator, data on foraging ecology and migratory patterns are essential to understanding the role of humpback whales as consumers in marine ecosystems.

Methods currently, or previously, used to study humpback whale ecology have had varying degrees of success. Photo-identification of individual whales has been widely used as a mark-recapture method for estimating population sizes and tracking movements (e.g. Calambokidis et al. 1997). Though this technique is relatively inexpensive and can produce reasonable estimates of abundance, it is limited by innate differences in the fluking behavior of individual whales and depends on resighting individuals on both breeding and feeding grounds. Satellite, acoustic, and radio telemetry have also been used to provide information on the movements and habitat use of large whales (Mate et al. 1995, Croll et al. 1998, Hooker & Baird 2001, Hooker et al. 2001, Baumgartner & Mate 2003), but these techniques are logistically difficult and can be prohibitively expensive. Recently, intrinsic methods involving the use of tissue assays have become practical to evaluate multiple aspects of cetacean ecology. Molecular markers are used to analyze population genetic structure and relatedness among cetacean populations on their breeding grounds (e.g. Baker et al. 1998), but do not describe feeding destinations or trophic ecology. Identifying fatty acids present in blubber assays can be used to distinguish prey use and habitat choice in marine mammals (e.g. Budge et al. 2006), but use of this technique on live whales is limited because tissue samples must penetrate deeper into the blubber layer than can currently be collected on free-ranging animals (Worthy and Samuel unpubl. data).

Fortunately, in recent years, the use of stable isotope analysis has also proved a valuable technique for providing information on trophic position, diet, and feeding

origins of migratory animals (Hobson 1999, Kelly 2000, Farmer et al. 2003). Isotopes are

atoms of the same element with different atomic weights due to different numbers of

neutrons. With respect to ecological studies, carbon and nitrogen are the two most

common isotopes analyzed. Carbon and nitrogen naturally occur in at least two stable

forms. Lighter forms, ^{14}N and ^{12}C, are more abundant than heavier forms, ^{15}N and ^{13}C.

Fractionation, or isotopic differences between the source and product, of stable isotope

ratios occurs when the lighter isotope is preferred in biochemical reactions. This

fractionation results in a step-wise enrichment, or increase in the concentration of the

heavier isotope relative to the standard for the element in question. As a result of

fractionation, the ratios of heavy to light isotope can be measured. The abundances of

nitrogen and carbon isotopes in animal tissues reflect the average isotopic composition of

the animal's assimilated diet (e.g. Deniro & Epstein 1978, Deniro & Epstein 1981, Rau et

al. 1983, Wada et al. 1987, Fry 1988). With respect to marine fauna, nitrogen

composition indicates relative trophic position (Fry 1988), while carbon reflects the

sources of primary production (Rau et al. 1983). For fasting species, tissues may be

enriched in ^{15}N, as these animals literally feed on themselves during the non-feeding

season (Cherel et al. 2005). Thus, the use of nitrogen ratios has shown considerable

promise as a diagnostic tool of body condition (Gannes et al. 1998).

Stable isotope analyses have been used to evaluate the trophic ecology of a

variety of marine mammal species (e.g. Todd et al. 1997, Gendron et al. 2001, Kurle &

Worthy 2001, 2002). Because stable isotopes can be extracted from skin, a standard

cetacean biopsy is an effective and non-invasive means to collect samples needed to

evaluate foraging ecology of free-ranging cetaceans (Todd et al. 1997, Gendron et al. 2001, Herman et al. 2005).

The goal of this dissertation is to increase our understanding of North Pacific humpback whale population structure and feeding ecology through analysis of stable carbon and nitrogen isotope ratios. The specific objectives of this research were to determine the stable isotope signatures of humpback whale feeding groups (Chapter 2), use stable isotope signatures to assign breeding humpback whales to a specific feeding aggregation and to describe their migratory patterns (Chapter 3), examine how nitrogen isotope values reflect difference in trophic position between feeding aggregations (Chapter 4), and finally to use stable isotopes to model a regional humpback whale diet (Chapter 5).

Chapter 2 of this dissertation, entitled "Population structure of North Pacific humpback whales on feeding grounds as shown by stable carbon and nitrogen isotope signatures," explores geographic variation in the stable isotope signatures of humpback whale skin collected from humpback whales in all known feeding areas within the North Pacific. The ratios used to define regional feeding groups then formed the basis for subsequent analyses of migration patterns and trophic relationships (Chapters 3 and 4).

Chapter 3, entitled "Using stable carbon and nitrogen isotope ratios to describe migratory movements of breeding North Pacific humpback whales," builds on results from Chapter 2. This chapter describes analysis of stable isotope signatures from individual humpback whales that were sampled on both their breeding and feeding grounds to determine the degree of change in the stable isotope signatures between

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habitats. Breeding whales that had not been sampled on feeding grounds were then assigned to one of the North Pacific feeding groups defined in Chapter 2.

Chapter 4, entitled "Differences in trophic position of North Pacific humpback whales as shown by stable nitrogen isotope ratios: implications on prey selection and resource quality," describes how the relative trophic position of the discrete feeding groups defined in Chapter 2 were determined by comparing $\delta^{15}N$ values of humpback whale skin to regional prey sources. Differences in trophic position between feeding groups were explored and discussed as potential indicators of survival and reproductive success.

Finally, Chapter 5 described how stable isotope ratios of humpback whale skin and prey resources were used to model the diet of humpback whales foraging near Kodiak Island, Alaska. A dietary mixing model was used to estimate the relative contributions of prey types to the humpback whale diet.

The studies described in the chapters of this dissertation represent the first attempt to apply stable isotope analysis to study cetacean ecology on both a broad and fine scale. Results from each chapter combine to provide new insights into the migratory movements, population structure, resource use, and foraging ecology of North Pacific humpback whales.

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Table 1.1. A summary of terms used within the text to describe groups of humpback whales.

Term	Definition
Stock	A management term used to define units of humpback whales for the purpose of estimating population parameters and human caused mortality; based primarily on location of breeding (Angliss & Outlaw 2008)
Feeding aggregation	A geographically separate group of foraging whales; little interchange occurs between aggregations
Feeding grounds	All known locations used by humpback whales for foraging; higher latitudes and usually occupied in the summer months
Breeding grounds	All known locations used by humpback whales for breeding, mating and calving; lower latitudes and usually occupied in the winter months
Population	All North Pacific humpback whales
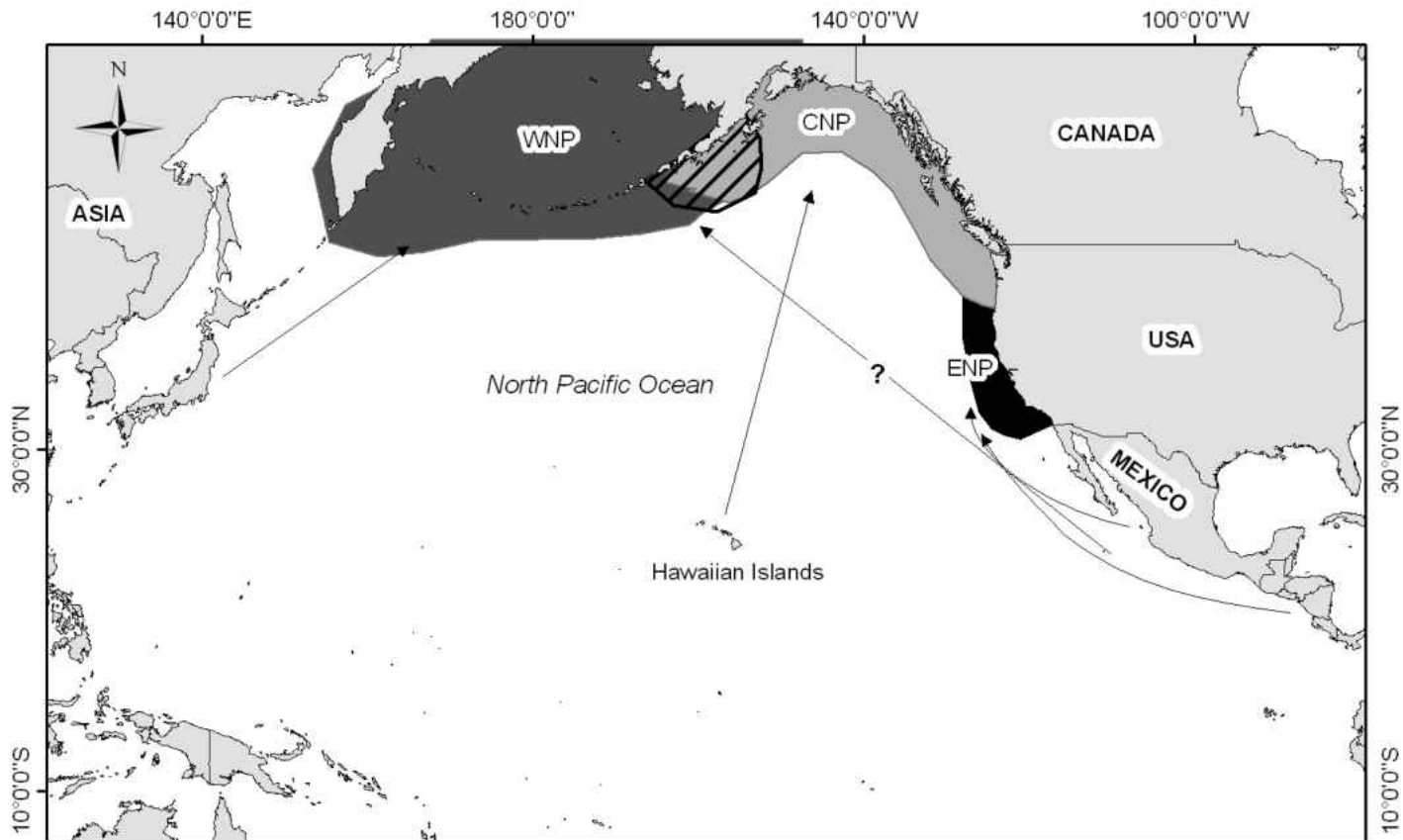
Figure 1.1. Map of the North Pacific Ocean showing the three stocks of humpback whales (shaded areas); Western North Pacific (WNP), Central North Pacific (CNP), and Eastern North Pacific (ENP). Arrows represent movements from southern breeding grounds. The stripe area indicates an area of potential overlap between WNP and CNP.

CHAPTER 2: POPULATION STRUCTURE OF NORTH PACIFIC HUMPBACK WHALES ON FEEDING GROUNDS AS SHOWN BY STABLE CARBON AND NITROGEN ISOTOPE RATIOS[1]

Introduction

Humpback whales (*Megaptera novaeangliae*) undergo one of the longest migrations of any mammal. Humpback whales spend the summer months foraging in productive high-latitude waters before migrating to lower latitudes to breed and give birth. During migration and while on mating and calving grounds, these whales will do little or no feeding (Dawbin 1966, Lockyer 1981b, Baraff et al. 1991, Laerm et al. 1997). As a result, humpback whale distribution includes seasonal usage of a number of different habitats creating diverse and complex habitat needs; one habitat must support extended bouts of foraging while the other must be suitable for mating and calving.

Within the North Pacific, humpback whales are known to breed in the waters of Asia, Mexico, Central America, and the Hawaiian Islands. Upon migration from breeding grounds, humpback whales segregate geographically into several discrete feeding aggregations, between which very little exchange occurs (Waite et al. 1999, Urbán R et al. 2000, Calambokidis et al. 2001, Witteveen et al. 2004). This pattern of movements means that whales feeding at one location may include individuals from multiple breeding grounds, creating a very intricate population structure (Calambokidis et al. 1996, Waite et al. 1999). This complexity, coupled with the inherent difficulty in studying pelagic marine mammals, has cofounded the description of

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[1]This manuscript is currently under review for publication in Marine Ecology Progress Series.

many demographic parameters for humpback whales, such as population structure, habitat use, and migration patterns.

One step in unraveling the complexity of humpback whale life history and habitat usage is to identify their foraging aggregations. Previous and on-going research have identified feeding aggregations in southeast Alaska, the California and Oregon coasts, Kodiak Island, and the Shumagin Islands (Straley 1994, Calambokidis et al. 1996, Baker et al. 1998, Waite et al. 1999, Calambokidis et al. 2001, Witteveen et al. 2004). Opportunistic sightings and historic whaling data suggest that additional feeding aggregations exist in other areas of the North Pacific, such as waters off of Russia and British Columbia, but a lack of dedicated research effort in these and other areas makes defining them difficult (Nishiwaki 1966, Ivashin & Rovnin 1967, Zerbini et al. 2006).

Traditionally, efforts to define humpback whale feeding aggregations have relied on mark-recapture techniques employing either identification photographs or genetic tissue assays. Both techniques are limited by the requirement that individuals be sampled on both habitats. Recently, stable carbon and nitrogen isotope ratios have been used in the analysis of migratory populations, specifically for studying aspects of population structure and feeding ecology (Hobson 1999, Kelly 2000, Farmer et al. 2003). The isotopic signatures of a consumer's tissues reflect the ratio of heavy to light isotopes in its foods.

Carbon isotope patterns result primarily from processes associated with photosynthesis, with changes in the ratio of heavy to light carbon isotopes ($^{13}C/^{12}C$) indicating sources of primary production. Marine systems are significantly enriched, or show a higher relative concentration, in ^{13}C compared to C_3 terrestrial systems due to the slower diffusion of carbon

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dioxide in water and the use of bicarbonate as a carbon source (Boutton 1991). In addition, stable carbon isotope ratios in marine systems have shown both a latitudinal gradient and benthic-pelagic continuum, which may result from fresh water influx and lighter stable carbon ratio values of phytoplankton (Fry 1981, Rau et al. 1982, Hobson et al. 1994). While carbon is an excellent predictor of location, ratios of nitrogen stable isotopes (^{15}N/^{14}N) provide a measure of relative trophic position. Nitrogen stable isotope ratios become less negative, or more enriched, with increasing trophic position due to the preferential excretion of ^{14}N in metabolic processes (Minagawa & Wada 1984). Geographically distinct patterns in both ratios have been used to investigate the migration patterns and rearing habitats of a number of species including birds, salmon, and sea turtles, as well as whales (Born et al. 2003, Kennedy et al. 2005, Baduini et al. 2006, Hobson 2006, Rocque et al. 2006, Caut et al. 2008). More recently, stable isotope analysis has been employed to classify migratory species by their feeding or breeding origins (e.g. Caccamise et al. 2000, Hebert & Wassenaar 2005b, a, Wunder et al. 2005, Szymanski et al. 2007).

The objectives of this study were to 1) use variation in isotopic carbon and nitrogen signatures of North Pacific humpback whales to describe distinct feeding groups and 2) use classification tree analysis to develop a predictive model to assign individuals to their foraging origins based on observed variation. Patterns in stable isotope signatures described in this study should be retained during migration and the breeding season as the result of humpback whale fasting behavior. As such, the model could be used to assign feeding destinations to animals sampled only on the breeding grounds, eliminating the need for a resampling event to confirm a migratory connection. Further, combining stable isotope analysis with other data sets, including

photo-identification and genetic markers, will provide a powerful set of tools useful in understanding the population structure and dynamics of North Pacific humpback whales.

Methods

Sample collection

Samples for isotopic analysis were collected from free-ranging humpback whales throughout all known feeding areas in the North Pacific basin as part of the Structure of Populations, Levels of Abundance and Status of Humpback whales (SPLASH) project. The SPLASH project was initiated in 2004 in an effort to collect photographs and tissue samples from humpback whales throughout their known range in the North Pacific basin. Effort was divided into 10 arbitrary sampling regions based on areas of pre-existing research effort, availability of researchers, or historic whaling records. Sampling regions were California and Oregon (CAOR), Washington and southern British Columbia (WASBC), northern British Columbia (NBC), southeastern Alaska (SEAK), northern Gulf of Alaska (NGOA), western Gulf of Alaska (WGOA), eastern Aleutian Islands (EAI), western Aleutian Islands (WAI), Bering Sea (BER), and Russia (Figure 2.1). Sampling occurred between 17 May and 4 December in 2004 and 22 April and 4 December 2005 (Calambokidis et al. 2008). In total, 5,604 samples were collected during SPLASH field efforts, of which 1,121 were made available for stable isotope analysis. Samples collected from animals identified as calves, juveniles, or dead (i.e. stranded) (n = 16) were immediately removed from analysis since it is not fully understood how samples from these categories may influence stable isotope ratios. An error during analysis of carbon

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meant one sample had a result for nitrogen only. Thus, a total of 1104 carbon and 1105 nitrogen samples were used for all analyses.

Samples were collected using a hollow-tipped biopsy dart fired by either a crossbow or modified .22 rifle. Darts collected the entire skin layer and a portion of the blubber layer, but did not sample any muscle. The preferred sampling location was the dorsal flank but samples were occasionally collected from the tail flukes. Skin that was sloughed following acrobatic displays (such as breaching and tail slapping) was also collected for analysis. At each sampling event, the date, location (latitude and longitude), group composition, and general whale behavior were recorded. In addition, identification photographs of tail flukes of sampled animals were collected whenever possible.

As soon as possible after collection, samples were preserved by either freezing or storage in dimethyl sulfoxide (DMSO) or ethanol. Though freezing was preferred, it was not always available at the more remote research locations and previous research has shown no significant difference when lipids are extracted from humpback whale and other cetacean skin when preserved in either DMSO or ethanol (Hobson et al. 1997, Todd et al. 1997, Marcoux et al. 2007).

Sample preparation and stable isotope analysis

A portion of skin from each sample (at least 10 mg wet mass) was sliced into small pieces to increase surface area and then oven-dried for 24 hours, followed by lipid extraction using petroleum ether in a Soxhlet extractor for an additional 24 hours (Dobush et al. 1985). Following lipid extraction, samples were again oven-dried at 60°C for 12 to 24 hours to evaporate off any remaining petroleum ether.

Dried, lipid-extracted samples were then ground to powder to ensure homogenization. Aliquots (0.7-1.5 mg) of homogenized sample were sealed in 5 mm by 9 mm tin capsules and then analyzed using a Finnigan MAT Delta Plus XL isotope ratio mass spectrometer (IRMS) at the University of Georgia Institute of Ecology Stable Isotope Laboratory.

Stable isotope ratios were 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 $^{13}C/^{12}C$. Standard reference materials were carbon from Pee Dee Belemnite and atmospheric nitrogen gas.

Quality assurance of stable isotope ratios was tested by running one known standard sample (bovine tissue) for each 12 unknown (humpback whale tissue) samples. Analytical errors for the bovine tissue ($n = 204$) were ± 0.1 (SD) for $\delta^{13}C$ and $\delta^{15}N$.

Statistical analysis

Data were tested for normality and homogeneity of variance using Kolmogorov-Smirnov and Levene's test, respectively. Sex was determined for a subset of sampled animals ($n = 590$) through genetic analysis (SPLASH unpubl. data). A preliminary analysis of variance (ANOVA), controlling for year and sampling region, to determine if sex influenced stable isotope ratios found no differences between males and females ($F_{1,554} = 1.5$, $p = 0.215$ for $\delta^{13}C$ and $F_{1,555} = 2.5$, $p = 0.128$ for $\delta^{15}N$). Thus, samples of known and unknown sex were pooled for the remainder of the analyses. Potential differences in the stable isotope ratios of animals that were sampled twice during the sample period were explored using paired sample t-tests.

Relationships between $\delta^{13}C$ and $\delta^{15}N$ and distance from shore, latitude, and longitude were explored through polynomial regression analysis. Akaike's Information Criteria (AIC) was used to determine the best fitting regression (Burnham & Anderson 2002). Distance from shore (in km) was calculated based on the distance from the sample location to the nearest coastline. Longitudes were standardized by using negative values to reflect degrees west of the prime meridian.

Classification

Classification tree analysis was used to determine the ability of stable isotope ratios to classify humpback whales to feeding regions. Classification trees are grown by repeatedly splitting the data via algorithms that partition the data into mutually exclusive groups (Breiman et al. 1984, De'ath & Fabricius 2000, StatSoft 2007).

Trees were constructed for analysis using sampling region as a categorical classification variable and $\delta^{13}C$ and $\delta^{15}N$ as independent variables. The isotope ratios were tested separately and then together, creating three potential classification models. To avoid under- or overfitting the data, a single, optimal tree in each model was selected as the simplest tree (smallest number of splits) with the highest predictive accuracy following methods developed by Breiman et al. (1984). The three optimal trees were then compared and the tree with the greatest explanatory power was selected as the best overall model for classification to sampling region. Following selection, the accuracy of the final model was assessed using cross-validation where 1/3 of the sample was withheld during initial analysis. The withheld data were then reclassified using the resultant model. This process was repeated three times. Finally, some sampling regions were combined to form isotopically similar feeding groups based on misclassification rates and

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geographic considerations. These feeding groups were then entered as the classification

variables and analyzed with the optimal classification tree.

All statistics were conducted within SPSS 15.0 or JMP 7.0 for Windows with a critical

value of $\alpha = 0.05$ for all analyses (Moran 2003). Values presented are mean ± SE.

Results

Isotopic values

Regional means for $\delta^{13}C$ ranged from a minimum of -18.8 ± 0.12 from WAI to a

maximum of -16.3 ± 0.05 from CAOR (Table 2.1). For $\delta^{15}N$, the minimum regional mean was

from WAI (11.4 ± 0.25) and the maximum regional mean was from CAOR (14.7 ± 0.09; Table

2.1). Kolmogorov-Smirnov tests of normality were significant for $\delta^{13}C$ (K-S = 0.033, $p = 0.006$)

and $\delta^{15}N$ (K-S = 0.030, $p = 0.019$). However, data were treated as normal due to a number of

factors: transformations failed to improve non-normal data, the K-S test can often give

significant results with respect to large sample sizes, and visual inspection of histograms and

normal Q-Q plots indicated normality (Field 2005).

Geographic variability

$\delta^{13}C$ varied quadratically with latitude ($F_{2,1101} = 225.4$, $r^2 = 0.29$, $p < 0.001$) and

longitude ($F_{2,1101} = 408.9$, $r^2 = 0.43$, $p < 0.001$); ΔAIC of linear and cubic relationships were >2,

indicating poorer fit than the quadratic regression (Burnham & Anderson 2002). The relationship

between $\delta^{13}C$ and distance was equally well-explained by linear, quadratic, and cubic models (all

AIC values were within 2), so the linear regression was selected as most parsimonious, although

the relationship was weak ($F_{1,1102} = 52.3$, $r^2 = 0.05$, $p < 0.001$; Figure 2.2). Model selections using $\delta^{15}N$ were similar; $\delta^{15}N$ varied quadratically with latitude ($F_{2,1102} = 164.7$, $r^2 = 0.23$, $p < 0.001$) and longitude ($F_{2,1102} = 106.4$, $r^2 = 0.16$, $p < 0.001$) and was linearly related (but weakly) to distance from shore ($F_{1,1103} = 27.1$, $r^2 = 0.02$, $p < 0.001$; Figure 2.2).

Individual variation

During the study period, 42 animals were sampled twice; 35 were sampled within the same year while the remaining seven were sampled in both 2004 and 2005. The mean values of $\delta^{13}C$ and $\delta^{15}N$ for animals sampled twice within the same year were not significantly different from one another ($t_{34} = -0.12$, $p = 0.908$ for $\delta^{13}C$ and $t_{34} = -0.24$, $p = 0.809$ for $\delta^{15}N$). Similarly, no significant difference between means of $\delta^{13}C$ ($t_6 = 1.33$, $p = 0.233$) or $\delta^{15}N$ ($t_6 = 1.39$, $p = 0.214$) were found for animals sampled in both 2004 and 2005. However, differences in the mean isotopic signatures of animals sampled in the same year but different sampling regions (3 of 35) were significantly different ($t_2 = 4.64$, $p = 0.043$ for $\delta^{13}C$ and $t_2 = 6.735$, $p = 0.021$ for $\delta^{15}N$).

Classification

The accuracy of the three classification models, shown by the percent of correct assignment to sampling region, was highest for the model that used both ratios as predictors (44.8% correctly classified), followed by the $\delta^{13}C$ only model (37.9%) and $\delta^{15}N$ only model (31.5%). The pattern was similar with respect to the explanatory power, with the dual isotope model showing the highest power ($R^2 = 0.32$), $\delta^{13}C$ following ($R^2 = 0.23$), and $\delta^{15}N$ with the

smallest ($R^2 = 0.14$). Thus, the optimal tree from the model using both isotope ratios was selected as best.

The cross-validated model correctly predicted sampling region for 45% of the samples. The model was able to correctly predict sampling region over 50% of the time for SEAK (64%), BER (63%), CAOR (54%), and NGOA (54%; Table 2.2). The number of correct classifications for the remaining regions fell below 50%. Regions often showed a majority of misclassifications to a single, adjacent region. WASBC samples were most frequently assigned to CAOR (23%), EAI to BER (61%), and WAI to RUSSIA (57%; Table 2.2; Figure 2.1).

Based on these results, the original 10 samples regions were combined to form six feeding groups. WASBC was combined with CAOR to form COW, and WGOA, EAI, and BER were combined to form CENT. Though WGOA was misclassified at similar rates to BER (39%) and NGOA (33%), WGOA samples were included in the CENT group due to the slightly higher value for BER. Finally, WAI was combined with RUSSIA to form WEST. NBC was not combined with any other sampling region due to the wide distribution of misclassified samples from this region. The six feeding groups differed significantly for both δ^{13}C (ANOVA, $F_{5,1098} = 102.9$, p<0.001) and δ^{15}N ($F_{5,1099} = 130.0$, $p<0.001$; Figure 2.3).

When applied to new feeding groups, results from the classification tree improved. The explanatory power of the model increased to 0.37 and accuracy to 57% after cross-validation. The highest rates of accurate classification were in COW (78%), SEAK (66%), and CENT (77%; Table 2.3). Misclassification rates for NBC were high (81% misclassified) and were again distributed among several feeding groups. The majority of misclassifications for WEST were assigned to SEAK (32%; Table 2.3). The accuracy of the model for NGOA was reduced from

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54% to 33%, with erroneous classifications attributed to both CENT (25%) and SEAK (24%; Table 2.3).

Random assignment correctly predicted feeding group membership 17% of the time on average, with distribution among feeding group as 16%, 12%, 21%, 18%, 26%, and 7% for COW, NBC, SEAK, NGOA, CENT, and WEST respectively. Thus, the classification tree for feeding groups performed 3.4 times better on average than random assignment with a range of 1.5 to 4.8.

Discussion

Geographic variability

Stable isotope ratios of carbon and nitrogen can be used to distinguish distinct feeding groups of humpback whales. Both $\delta^{13}C$ and $\delta^{15}N$ varied significantly with respect to latitude, longitude, and distance from shore of sample collection.

Numerous previous studies have explored latitudinal gradients in $\delta^{13}C$, most of which have found that mid latitudes tend to be more enriched in $\delta^{13}C$ than higher latitudes (Rau et al. 1982, Goericke & Fry 1994). Results presented here are somewhat contrary. Values of $\delta^{13}C$ at the highest latitudes (NGOA, SEAK, and RUSSIA) were not the most depleted as would be expected, indicating a quadratic, and not linear, relationship between latitude and carbon stable isotope ratios (Figure 2.2). The mean $\delta^{13}C$ values of humpback whale skin from NGOA were on par with previous studies, but values from CENT seemed to be relatively more depleted (Hobson et al. 1997, Kurle & Worthy 2001, 2002). Thus, the deviation in the expected linear pattern may be driven by effects in the CENT sampling regions, such as fresh water influence or

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anthropogenic sources of carbon. Another explanation is that latitude was not the only factor determining the distribution of ^{13}C in North Pacific humpback whales.

Other known δ^{13}C gradients may explain the spatial variation in stable carbon isotope ratios, which are generally lower in pelagic (offshore) food webs than benthic (near-shore) food webs (McConnaughey & McRoy 1979, Hobson 1993, Burton & Koch 1999). Most samples in SEAK and NGOA were collected in near-shore habitats and had higher δ^{13}C values than could be explained by latitude alone. Similarly, a significant portion of NBC and CENT samples were collected either off the continental shelf or very near the edge and were more depleted than those collected on the shelf. Depletion in samples collected in close proximity to or off the shelf edge may exhibit stable carbon isotope ratios indicative of pelagic food webs. Thus, distance from the shelf edge, rather than distance from shore, may have a stronger influence on stable carbon isotope ratios. Regardless, it is clear that the stable carbon isotope ratio of North Pacific humpback whale skin was likely determined by the interplay between latitudinal, benthic versus pelagic and perhaps most importantly longitudinal food web gradients (see below).

The δ^{15}N values of humpback whale skin varied quadratically with increasing latitude as well (Figure 2.3). Wada & Hattori (1991) suggested latitudinal gradients in the δ^{15}N values of phytoplankton were the result of low concentration of ammonia and nitrite in tropical areas, but Rubenstein & Hobson (2004) stated that the reasons for ^{15}N enrichment with increasing latitude were unclear. Differences in nitrogen signatures may also be attributed to trophic position since δ^{15}N increases between 2‰ and 5‰ with each trophic level (Peterson & Fry 1987, Post 2002). Humpback whales are classified as generalists, foraging on both fish and zooplankton (Nemoto & Kasuya 1965, Nemoto 1973, Perry et al. 1999), but regional differences in prey choice may

impact relative trophic positions of feeding groups. However, the cause of differences in δ^{15}N cannot be determined without first establishing the δ^{15}N value at the base of regional food webs (Post 2002). If these baseline data vary little between sampling regions, our results indicate that animals belonging to the COW fed at the highest trophic level (primarily fish), followed by NGOA, with the remaining groups all feeding at a similar lower level (primarily zooplankton) (Lesage et al. 2001, Das et al. 2003).

The quadratic relationships between both δ^{13}C and δ^{15}N and longitude contradicted previously observed relationships. Previous studies found no longitudinal effect on δ^{15}N, but a strong increase in δ^{13}C from east to west (Saupe et al. 1989, Schell et al. 1998, Knoche et al. 2007). The large size of our study area may have contributed to this difference. Increasing stable carbon isotope ratios from east to west have been attributed to fresh water inputs and areas of lower salinity within study areas that are relatively small in scale when compared to the entire North Pacific Ocean (Naidu et al. 1993, Schell et al. 1998). In such studies, sources of fresh water input may be identified as a single river basin, but given the breadth of our study area it is not possible to identify all of the sources that may be driving the observed pattern. Regardless of cause, the isotopic ratio of carbon, and to a lesser extent nitrogen, in humpback whale skin varied significantly with longitude.

<center>Individual variation</center>

The stable isotope ratios of twice-sampled animals can be predicted based on known patterns of stable isotope ratios in foraging animals. If sequential sampling of an individual occurs within the same sampling region, isotopic signatures, carbon in particular, should remain

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relatively constant. If the animal moves to a different feeding area between sampling events, however, changes in the stable isotope ratios should be detectable. Exploration of twice-sampled humpback whales in this study supports both predictions. When both sampling events occurred in the same sampling region, $\delta^{13}C$ and $\delta^{15}N$ were not significantly different, regardless of whether sampling events occurred in the same or sequential years. In contrast, whales sampled in different feeding regions showed significant differences in these ratios. These results lend considerable support to the use of stable isotope ratios as descriptors of foraging locations.

Classification

Classification trees have a number of advantages over discriminant function analysis and linear regression, both of which are often used in stable isotope assignment studies. Classification trees represent a modern statistical technique well suited for modeling ecological; data model output is hierarchical and based on logical if-then conditions and are both nonparametric and nonlinear (De'ath & Fabricius 2000, Spruill et al. 2002, StatSoft 2007). Classification tree analysis was able to assign 57% of the humpback whale tissue samples to the correct feeding group in the best performing model (Table 2.3). In this tree, groups were classified using both $\delta^{13}C$ and $\delta^{15}N$. The best model correctly classified all six feeding groups over three times higher on average than random assignment and nearly five times higher for two of the groups. Classification was lowest for NBC, NGOA, and WEST, with misclassifications occurring at rates greater than 50%.

There are a number of potential reasons behind misclassifications. First, similarities in the sampling position between regions may result in misclassifications due to latitudinal or longitudinal effects on stable isotope ratios. Latitudinal similarities may explain the high

classification of NBC samples to CENT since the mean sampling latitude differed by only two degrees on average between these two groups.

Additional misclassification could be explained if a feeding group does not truly represent a distinct feeding destination for North Pacific humpback whales, but rather a transitional area for animals en route to other feeding grounds. Wide distribution of misclassification to other feeding groups, such as the distribution of misclassifications for NBC, may indicate a transitional area. Also, boundaries between feeding groups may not be exactly as described. For example, the boundary between CENT and NGOA may actually lie within the WGOA and samples from this region should be divided amongst these feeding groups rather than assigned exclusively to WEST.

Finally, small sample size or high variability may account for misclassifications. The RUSSIA and WAI sampling regions, which together comprised the WEST feeding group, showed some of the highest regional variability in stable isotope ratios and the lowest sample sizes, which may have contributed to the large misclassification rates for WEST. Whether this high variability was merely the result of smaller sample size is unclear, but increased future sampling efforts in these regions may help elucidate the potential influences on isotopic variability to help improve classification for this feeding group.

The time frame of the diet estimated from stable isotope ratios depends on tissue turnover rates. The turnover rate of humpback whale skin has never been measured, but a turnover rate of approximately 7 to 14 days has been estimated (Todd 1997). However, turnover was not likely to play a significant role in the analyses presented here. The turnover rate of humpback whale skin

should not influence stable isotope ratios if animals are using the same feeding groups throughout the feeding season, which was a primary assumption of this study.

Overall, ratios of carbon and nitrogen stable isotopes found in humpback whale skin showed considerable promise for distinguishing feeding groups of North Pacific humpback whales. The ability of a multiple-isotope classification tree to determine feeding location has far-reaching implications. Beyond defining distinct feeding groups, geographic differences in stable isotope ratios of both humpback whales and their potential prey can be used to explore the foraging ecology and prey use within regional food webs. The ability to describe differences in diets may contribute to the understanding of prey selection and specialized foraging behaviors between and among regions. Perhaps more importantly, the classification model may be able to identify the feeding destination of humpback whales while they are fasting on their breeding grounds. Successful use of the model in this application provides a new method of describing the migratory movements of humpback whales without the need for a resighting or resampling event. Using stable isotopes to classify feeding location and explore regional diets can help elucidate how choice of prey or foraging location dictates animal health and, in turn, contributes to the relative success on breeding grounds. This technique would clearly be applicable to the many other migratory populations whose benefits of foraging are carried over to breeding grounds.

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Table 2.1. Sample totals and the number of known females and mails for each of 10 sampling regions sampled as a part of the SPLASH project. Mean values (±S.E.) for δ^{13}C and δ^{15}N with minimum and maximum values for each region are also shown.

Region	N	Females	Males	δ^{13}C	δ^{15}N
RUSSIA	67	37	25	-17.7 ± 0.1 -19.5, -15.9	12.5 ± 0.22 8.6, 16.1
BER	122	56	50	-18.5 ± 0.04 -19.6, -15.9	12.4 ± 0.1 7.4, 15.7
WAI	14	5	7	-18.8 ± 0.12 -19.5, -18.1	11.4 ± 0.25 10.1, 13.4
EAI	56	24	26	-18.5 ± 0.07 -19.6, -17.5	12.1 ± 0.16 9.1, 14.9
WGOA	104	39	27	-18.5 ± 0.08 -23.0, -15.8	13.1 ± 0.08 11.3, 15.3
NGOA	199	47	44	-17.6 ± 0.05 -20.2, -15.9	13.6 ± 0.07 8.8, 16.2
SEAK	227	23	5	-17.2 ± 0.05 -21.2, -15.4	12.7 ± 0.06 7.8, 15.1
NBC	135	1	3	-17.7 ± 0.06 -20.0, -15.9	13.0 ± 0.08 10.6, 15.8
WASBC	53	17	29	-16.8 ± 0.08 -18.8, -15.9	14.6 ± 0.13 11.2, 15.9
CAOR	128	55	70	-16.3 ± 0.05 -17.9, -15.2	14.7 ± 0.09 11.8, 16.6
Total	1105	304	286	-17.6 ± 0.03	13.2 ± 0.04

Table 2.2. Classification results produced by classification tree analysis of δ^{13}C and δ^{15}N (‰) as predicting variables for humpback whale skin collected from 10 sampling regions.

Known Sampling Region	Predicted Sampling Region											
	RUSSIA	BER	WAI	EAI	WGOA	NGOA	SEAK	NBC	WASBC	CAOR	Total	% Correct
RUSSIA	18	2	0	0	0	20	24	3	0	0	67	27%
BER	12	77	0	0	6	19	3	5	0	0	122	63%
WAI	8	5	0	0	0	1	0	0	0	0	14	0%
EAI	10	34	0	0	0	9	1	2	0	0	56	0%
WGOA	0	39	0	0	23	33	2	6	0	1	104	22%
NGOA	3	23	0	0	3	107	31	18	12	1	198	54%
SEAK	2	29	0	0	0	34	145	12	5	0	227	64%
NBC	5	40	0	0	0	31	23	30	6	0	135	22%
WASBC	0	1	0	0	0	9	6	1	24	12	53	45%
CAOR	0	0	0	0	0	10	36	0	13	69	128	54%
											Overall	45%

Table 2.3. Classification results produced by classification tree analysis of δ^{13}C and δ^{15}N (‰) as predicting variables for humpback whale skin collected from six feeding groups. Feeding groups were formed based on misclassification of sampling regions in preliminary classification tree analysis.

	Predicted Feeding Group							
Known Feeding Group	WEST	CENT	NGOA	SEAK	NBC	COW	Total	% Correct
WEST	28	14	12	26	0	1	81	35%
CENT	19	218	17	15	12	1	282	77%
NGOA	1	50	66	47	16	18	198	33%
SEAK	3	28	24	150	8	14	227	66%
NBC	2	44	21	35	25	8	135	19%
COW	1	3	18	14	3	142	181	78%
							Overall	57%
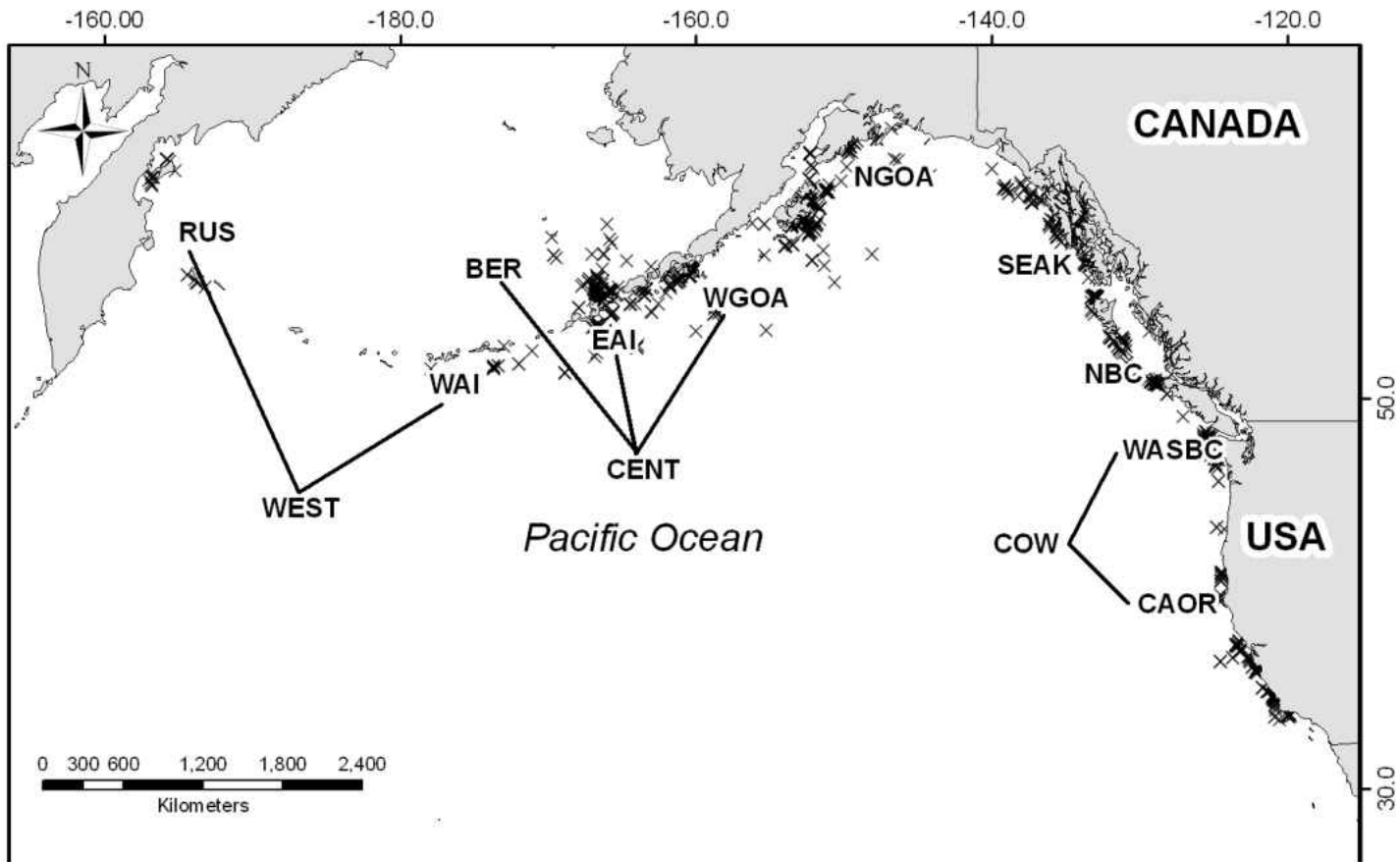
Figure 2.1. Map of the North Pacific Ocean showing the 10 sampling regions of the SPLASH project. Lines drawn from sampling regions indicate consolidated feeding groups. Sampling locations are also shown (x).
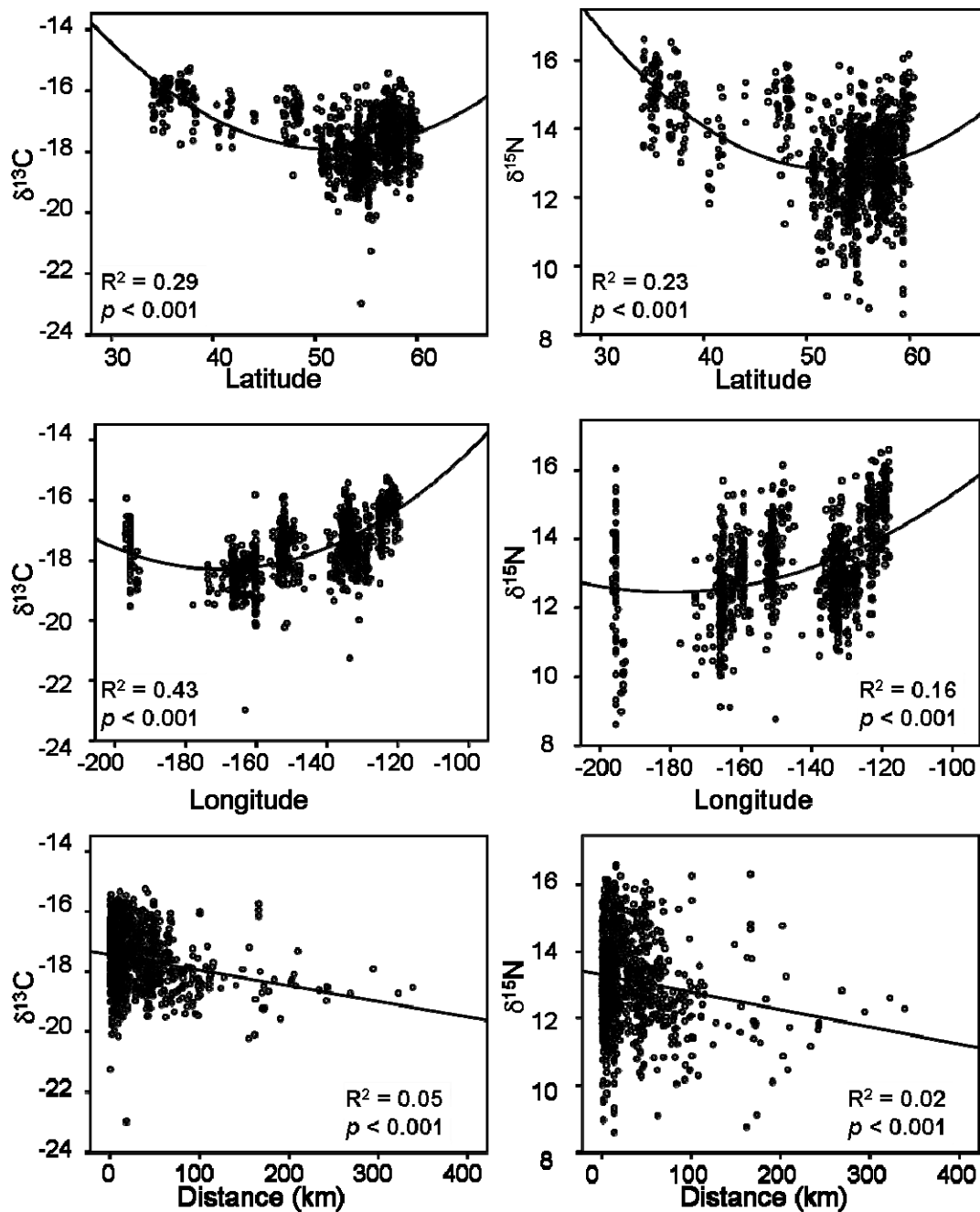
Figure 2.2. Relationships of $\delta^{13}C$ (‰) and $\delta^{15}N$ (‰) of the skin of North Pacific humpback whales to latitude, longitude and distance from shore (km) of sample collection. Regression results are also shown.
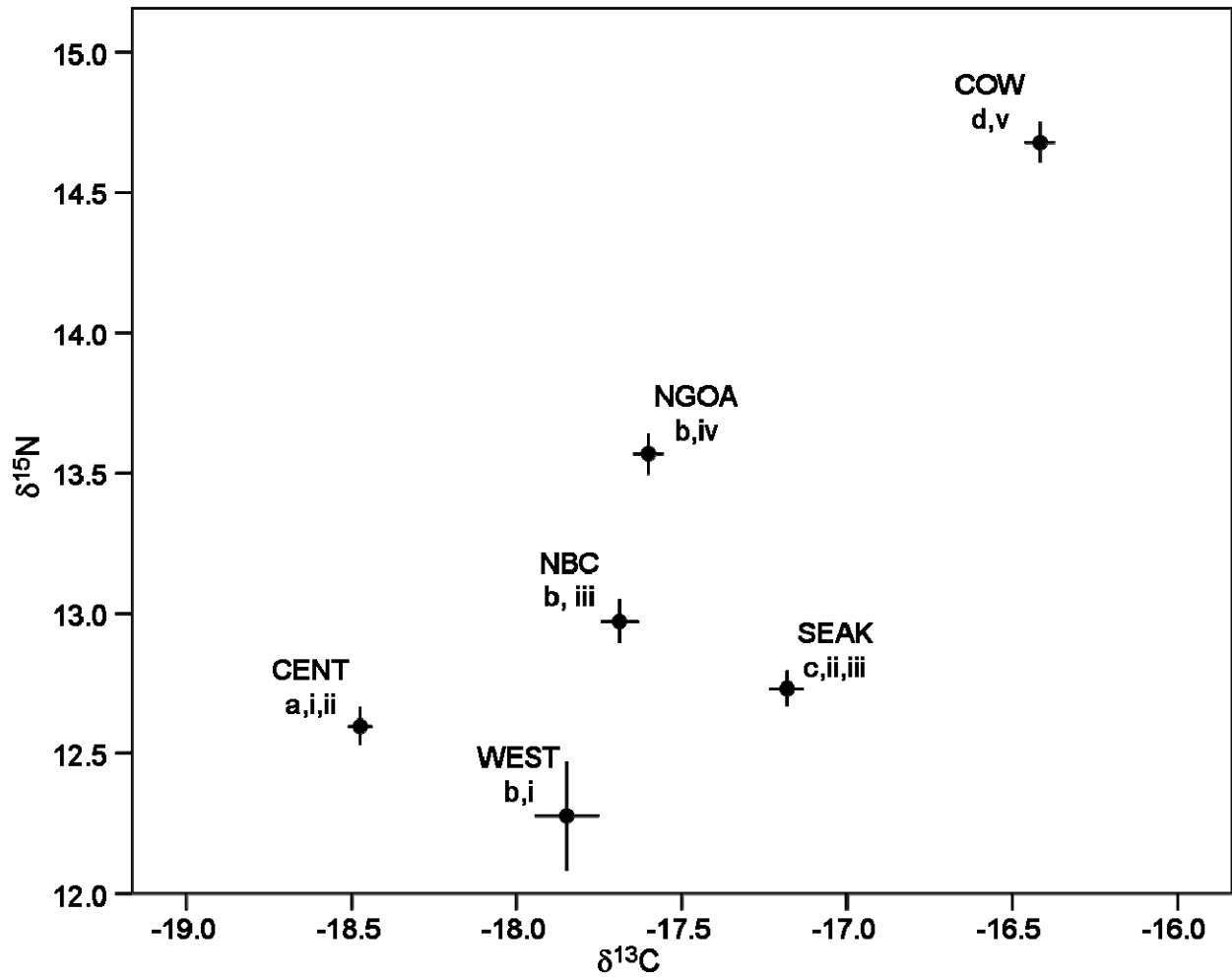
Figure 2.3. Mean values (± SE) of $\delta^{15}N$ and $\delta^{13}C$ (‰) for each of the six feeding groups. Letters indicate feeding groups with similar $\delta^{13}C$ means, while Roman numerals indicate feeding groups with similar $\delta^{15}N$ means.

CHAPTER 3: USING STABLE CARBON AND NITROGEN ISOTOPE RATIOS TO DESCRIBE MIGRATORY MOVEMENTS OF BREEDING NORTH PACIFIC HUMPBACK WHALES

Introduction

Migration has evolved independently among a number of animal taxa, including birds, ungulates, and marine mammals. Large baleen whales undergo seasonal migrations in order to take advantage of seasonal peaks in prey abundance (Corkeron & Connor 1999). The North Pacific humpback whale (*Megaptera novaeangliae*) is one species of baleen whales that practices this behavior, spending the summer months foraging in cool, productive waters before migrating to lower latitudes for mating and calving, where they do little or no feeding (Dawbin 1966, Lockyer 1981b, Baraff et al. 1991, Laerm et al. 1997).

Humpback whales segregate into geographically distinct aggregations while on their feeding grounds. While very little exchange occurs between these aggregations, several aggregations may converge on a common breeding ground (Calambokidis et al. 1996, Waite et al. 1999, Urbán R et al. 2000, Mizroch et al. 2004). Although not exact, some migratory patterns of humpback whales have been described. Broadly, humpback whales wintering in the Hawaiian Islands migrate to waters off Alaska (Baker et al. 1990, Perry et al. 1990, Calambokidis et al. 1997); humpback whales using Japanese waters for winter habitat migrate to Russia and the Bering Sea (Berzin & Rovnin 1966, Nishiwaki 1966, Darling 1991); finally those breeding near coastal Mexico migrate along the west coast of North America to destinations between California and southern British Columbia (Calambokidis et al. 1989, Steiger et al. 1991, Calambokidis et al. 1993). Humpback whales from a breeding ground offshore Mexico

(Revillagigedos Islands) migrate to an as yet unknown foraging location, though some animals have been sighted in the western Gulf of Alaska (Witteveen et al. 2004).

The life history of North Pacific humpback whales is, therefore, quite complex and many questions remain about their population structure. As an endangered species, unanswered questions about migratory destinations, routes, and habitat usage inhibit management and conservation efforts. Research focused on linking disparate habitats is needed in order to address this issue. Traditional techniques used to identify migratory connections, including photo-identification (i.e. Urbán R et al. 2000, Calambokidis et al. 2001) and genetic markers (i.e. Baker et al. 1986), are limited by a dependence on resighting or resampling individuals or the cost of analysis. Fortunately, the analysis of stable carbon and nitrogen isotope ratios has emerged as a useful tool for exploring habitat connectivity in migratory animals, including seabirds, shorebirds, elephants, pinnipeds and cetaceans (Best & Schell 1996, Farmer et al. 2003, Aurioles et al. 2006, Cerling et al. 2006, Cherel et al. 2006, Furness et al. 2006). Stable isotope analysis is relatively inexpensive, allows for sampling of free-ranging animals, and requires very little tissue, and is thus fairly non-invasive. Stable isotope analysis can be used in migratory studies because the stable isotope signatures of an animal's tissues reflect that of its regional food web (Peterson & Fry 1987, Schell et al. 1989a, b). Animals moving between isotopically distinct food webs should retain information from their previous foraging location (Hobson 1999). In the case of humpback whales, which do not feed on the breeding grounds, isotopic signatures of foraging grounds should be retained throughout the breeding season.

In this study, results from previous analysis of carbon and nitrogen stable isotope signatures of foraging humpback whales were applied to investigate relationships of animals

sampled on breeding grounds and to assign breeding animals to a feeding group. Results provide insight into the intricate population structure and ecology of North Pacific humpback whale populations without having to sample or photograph the same animal on both habitats.

Methods

Sample Collection

Samples for isotopic analysis were collected from free-ranging humpback whales throughout all known breeding regions in the North Pacific basin as a part of the Structure of Populations, Level of Abundance, and Status of Humpback whales (SPLASH) project. Effort was divided into four sampling regions. Sampling regions were defined based distribution of humpback whale on breeding grounds, areas of pre-existing research effort and availability of researchers. Sampling regions were defined as Asia, Hawaii (HI), Mexico (MEX), and Central America (CENT AM) (Figure 3.1). Since sampling regions were fairly broad, breeding areas within some sampling regions were defined by SPLASH protocol. Sample collection occurred on five Hawaiian Islands, but comprised just a single breeding area. For MEX, breeding areas were the Baja Peninsula (Baja Pen), mainland Mexico (Main Mex) and the offshore Revillagigedos Islands (Rev Is) and for Asia, the areas were Ogasawara and Okinawa, Japan and the Philippines. Though Costa Rica, Nicaragua, and Guatemala all served as sampling locations within CENT AM, small sample sizes resulted in their consideration as a single breeding area (Cent Am; Figure 3.2).

Sampling effort occurred between 09 January and 01 May for the 2004 breeding season, 19 December 2004 and 13 May for the 2005 breeding season, and 10 January and 01 May for the 2006 breeding season (Calambokidis et al. 2008).

Sample collection and preservation followed methods detailed in Witteveen et al. (*In review*). Briefly, skin samples were collected using a biopsy darting system or following acrobatic displays (such as breaching and tail slapping). Whenever possible photographs of the tail flukes of sampled individual were also collected at each sampling event. Additional data recorded included the date, location (latitude and longitude), and general whale behavior.

<center>Sample preparation and stable isotope analysis</center>

Skin samples were prepared for stable isotope analysis through a multi-step process that included oven drying, extraction of lipids, and homogenization (Chapter 2, Witteveen et al. *In review*). Samples were analyzed for stable carbon and nitrogen isotope ratios using a Finnigan MAT Delta Plus XL isotope ratio mass spectrometer (IRMS) at the University of Georgia Institute of Ecology Stable Isotope Laboratory.

Stable isotope ratios were 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 $^{13}C/^{12}C$. Standard reference materials for ^{15}N and ^{13}C were atmospheric N_2 gas and Pee Dee Belemnite, respectively. Analytical errors were ± 0.1 for both $\delta^{13}C$ and $\delta^{15}N$.

Statistical Analysis

Data were tested for normality and homogeneity of variance by each region using Kolmogorov-Smirnov and Levene's test, respectively.

Differences in stable isotope ratios were tested using factorial analysis of variance (ANOVA), run separately for $\delta^{13}C$ and $\delta^{15}N$. Factors explored were sampling region and year The sex of 202 individuals was known (60 females and 142 males). Sexes did not differ for either carbon ($F_{1,200} = 0.19$, $p = 0.664$) or nitrogen ($F_{1,200} = 0.01$, $p = 0.913$) ratios when controlling for sampling region and year, so sex was excluded as a factor in subsequent analyses. Breeding areas within ASIA and MEX as defined by SPLASH were also explored to determine if finer scale differences were present within these sampling regions. Finally, differences between breeding areas for $\delta^{13}C$ and $\delta^{15}N$ without consideration to sampling region were analyzed.

The relationship between breeding and feeding ratios was explored for individual whales that were sampled on both grounds through simple linear regression analysis, with breeding δX as the dependent variable and feeding δX and time between samples as predictor variables. In addition, ratios were compared using paired sample t-tests to determine if measured stable isotope ratios were significantly different between the two habitats. Photographs of the ventral side of the flukes of these animals identified them as the same individual at both locations.

A model constructed to classify isotopically distinct feeding groups of North Pacific humpback whales using classification tree analysis was applied to breeding area samples (Chapter 2, Witteveen et al. *In review*). The classification model incorporated $\delta^{13}C$ and $\delta^{15}N$ as variables to predict foraging location for animals sampled on feeding grounds. Feeding groups

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were defined as COW, NBC, SEAK, NGOA, CENT, and WEST (Chapter 2, Witteveen et al. *In review*). The model was applied to breeding samples in order to determine the success of the model at assigning individuals to one of the six feeding groups. This analysis was based on the assumption that the stable isotope ratios of breeding, and therefore fasting, humpback whales reflect location of foraging. The model was first tested by applying it to samples of known feeding origin and was then applied to all samples in the data set. Assignments of breeding animals to feeding groups based on classification tree analysis were compared to photographic matches resulting from SPLASH analysis as a means of testing classification results versus real-world data.

All statistics were conducted within SPSS 15.0 or JMP 7.0 for Windows with a critical value of $\alpha = 0.05$ for all analyses (Moran 2003). Values presented are mean ± SE. Homogeneous subsets were determined through Tukey's post-hoc tests following all analyses.

Results

Stable isotope ratios in 597 samples collected by the SPLASH project over three years and between each of the breeding regions were analyzed. Tests of normality were significant for $\delta^{13}C$ (Kolmogorov-Smirnov K-S = 0.058, $p < 0.001$) and $\delta^{15}N$ (K-S = 0.067, $p = 0.001$) for all samples combined, indicating that these data did not follow a normal distribution. Results varied when each region was tested separately. Only MEX differed from normality both $\delta^{13}C$ (K-S = 0.102, $p = 0.004$) and $\delta^{15}N$ (K-S = 0.09, $p = 0.02$). HI samples deviated significantly from a normal distribution for $\delta^{15}N$ (K-S = 0.072, $p<0.001$), but not $\delta^{13}C$ (K-S = 0.042, $p = 0.2$). The same results were seen for ASIA (K-S = 0.12, $p<0.001$ for $\delta^{15}N$ and K-S = 0.065, $p = 0.2$), but

were opposite for CENT AM (K-S = 0.117, p = 0.2 for δ^{15}N and K-S = 0.125, p = 0.179 for

δ^{13}C). Despite the significant K-S test results, data were treated as normal due to a number of

factors: transformations failed to improve non-normal data, the K-S test can often give

significant results with respect to large sample sizes, visual inspection of histograms and normal

Q-Q plots showed normality, and general linear models are generally considered robust to

departures from normality (Field 2005).

Efforts were made to analyze equal numbers of samples from each region, but this was

not always possible due to variability in sampling effort. The Hawaiian Islands experienced the

greatest amount of effort, which is reflected in the larger sample size for this region (Table 3.1).

For all sampling regions combined, the mean value of δ^{13}C was -17.8 ± 0.04 and the mean value

of δ^{15}N was 12.9 ± 0.06 (Table 3.2). Regional mean values of δ^{13}C ranged from a high of -16.3 ±

0.14 for CENT AM to a low of -18.3 ± 0.06 for ASIA. This pattern held for nitrogen values; the

highest mean was CENT AM (14.9 ± 0.13) and lowest was ASIA (12.1 ± 0.13).

δ^{13}C values were significantly affected by sampling region ($F_{3,585}$ = 62.3, p<0.001) and

year ($F_{2,585}$ = 4.2, p = 0.016), but not the interaction between the two ($F_{6,585}$ = 1.9, p = 0.07).

With respect to δ^{15}N, only sampling region was significant ($F_{3,585}$ = 37.2, p<0.001), while year

($F_{2,585}$ = 1.5, p = 0.21) and the interaction ($F_{6,585}$ = 1.2, p=0.33) were not. Sampling region was

grouped into three homogenous subsets with respect to both carbon and nitrogen stable isotope

ratios. For δ^{13}C, ASIA and HI were not significantly different from one another, while MEX and

CENT AM were distinct from all sampling regions. CENT AM and ASIA were distinct with

respect to δ^{15}N, while HI and MEX could not be distinguished (Figure 3.3).

Significant differences in δ^{13}C for ASIA breeding areas were seen ($F_{2,134} = 10.3$ $p<0.001$) with the Philippines and Okinawa creating one group separate from Ogasawara. The three ASIA breeding areas were not significantly different from one another with respect to δ^{15}N ($F_{2,134} = 0.5$, $p = 0.59$). Within MEX, breeding areas differed for both δ^{13}C and δ^{15}N ($F_{2,114}$ 8.1, $p < 0.001$ and $F_{2,114}$ 5.5 $p = 0.005$ respectively). Post-hoc tests for δ^{13}C separated the Rev Is and Baja Pen from Main Mex, but not from one another. Slightly different groupings were seen in δ^{15}N, with Baja Pen grouped with both Rev Is and Main Mex with the latter two separating from one another.

Carbon stable isotope ratios were significantly different between breeding areas when sampling region was not considered ($F_{7,589} = 34.9$, $p <0.001$), though similar groupings did follow regional patterns. Post-hoc tests produced five homogeneous subgroups (Figure 3.3). Group one included Philippines and Okinawa, group two contained Ogasawara and Hawaiian Is, group three contained Hawaiian Is. with Rev. Is., which was also grouped with Baja Pen. in the fourth group. Finally, Main Mex and Cent Am made up the fifth and final subgroup with respect to δ^{13}C. ANOVA was significant for δ^{15}N as well ($F_{7,589} = 18.9$, $p<0.001$), though differentiation between breeding areas was not as definitive as with δ^{13}C (Figure 3.3).

Forty-four individuals were sampled on both feeding and breeding grounds. Of these, 27 were sampled on their feeding ground prior to sampling on the breeding ground, while the remaining 17 were sampled on their breeding grounds first. Regression analysis showed a significant positive relationship between breeding δ^{15}N and feeding δ^{15}N ($F_{1,42} = 31.3$, $r^2 = 0.43$, $p<0.001$). With respect to stable carbon isotope ratios, there was also significant positive relationship between the two carbon ratios ($F_{1,42} = 2.1$, $r^2 = 0.09$, $p = 0.05$). Adding time,

defined as the number of days between sampling events, as a factor in analyses did not improve

regression results for $\delta^{15}N$ ($F_{2,41} = 15.3$, $r^2 = 0.43$, $p < 0.001$) or $\delta^{13}C$ ($F_{2,41} = 2.6$, $r^2 = 0.11$ $p =$

0.083l; Figure 3.4). Results from paired t-tests showed that there was no significant difference

between breeding $\delta^{15}N$ and feeding $\delta^{15}N$ ($t_{43} = 1.57$, $p = 0.123$) or the $\delta^{13}C$ ratios ($t_{43} = -0.71$, $p =$

0.481).

The classification tree model based on $\delta^{13}C$ and $\delta^{15}N$ was applied to foraging animals and

breeding animals of known feeding group and resulted in successful assignment of 56% of cases.

Correct assignment to feeding groups based on chance alone was only 17%. Thus, the

classification tree was 3.3 times more successful at feeding group assignment. Assignments to

feeding groups were summarized by sampling region and breeding area. The highest proportion

for each sampling region was as follows: ASIA to WEST (38%), HI to CENT (36%), MEX to

COW (31%), and CENT AM to COW (79%). Distribution of assignments among breeding areas

ranged from 80% of Cent Am to the COW feeding group to 0% for several of breeding

area:feeding group comparisons (Figure 3.5). Strong connections were seen between Philippines

and CENT (57%), Okinawa to WEST (48%), and Baja Pen, Main Mex and Cent Am to COW

(34%, 60%, and 80% respectively) (Figure 3.5).

Assignments of breeding animals to feeding grounds based on classification tree analysis

of stable isotope ratios differed by 12% on average from photographic matches of individuals

between breeding and feeding grounds. Some breeding areas exhibited strong average agreement

between classification tree and photographs, such as Baja Pen, Main Mex and Cent Am, which

differed by only 8%, 7% and 7% respectively. The highest discrepancies were found for

Philippines (24%) and Rev. Is. (14%). In some cases, classification tree assignment was nearly

identical to result from matching. For example, for animals sampled in Rev Is, 11% were assigned to the NBC feeding groups as per classification tree analysis; a difference of only 2% from the 9% of Baja Pen photographs that were matched to that feeding group (Figure 3.5).

Discussion

Analysis of δ^{13}C and δ^{15}N in humpback whale skin proved a useful method for determining the feeding destinations of breeding whales. Results were generally in agreement with current knowledge of stable isotope ecology and with previous exploration of this population in their feeding groups (Chapter 2, Witteveen et al. *In review*). Broadly, results of our analysis can be explained by the fact that the stable carbon isotope ratio reflects feeding origins and sources of primary productivity and nitrogen ratios describe relative trophic positions (Fry 1981, Hobson & Welch 1992, Rau et al. 1992, Post 2002). Humpback whales do not feed to any significant extent while on their breeding grounds (Dawbin 1966, Lockyer 1981b, Baraff et al. 1991, Laerm et al. 1997) and, as such, the ratio of δ^{13}C should preserve the location of most recent foraging while δ^{15}N shows the trophic level of foraging (Gannes et al. 1997, Hobson 1999, Kelly 2000, Post 2002, Hobson 2006, Rocque et al. 2006). Our results suggest very little change in the ratios between habitats and support the assumption that δ^{13}C and δ^{15}N of breeding animals remain relatively static until foraging resumes. It has also been hypothesized that differences in δ^{15}N between feeding and breeding groups may reflect fasting (Hobson et al. 1993, Cherel et al. 2005). Ratios of ^{15}N are frequently used as an indicator of relative trophic position, with δ^{15}N becoming enriched by 3 to 4‰ with each trophic level in a food web (Hobson et al. 1994, Post 2002). While on breeding grounds, a humpback whale is surviving on blubber

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reserves accrued during foraging and is essentially feeding on itself, which would be a higher

trophic level than the fish or zooplankton of a typical diet. Therefore, if stable nitrogen isotope

ratios of breeding animals did reflect fasting behavior, they should be significantly more

enriched than $\delta^{15}N$ of feeding animals. This phenomenon was not observed here. While a

relatively strong positive relationship was found between the two nitrogen stable isotope ratios,

the values themselves were not significantly different. Other studies have looked for patterns of

enrichment in $\delta^{15}N$ of fasting mammals and birds and have similarly found a lack of enrichment

(e.g., Hobson & Schell 1998, Ben-David et al. 1999, Williams et al. 2007) It is possible that

animals experiencing regular bouts of fasting have adapted to this behavior and are prevented

animals from becoming "nutritional stressed." As such, enrichment and significant changes in

$\delta^{15}N$ may only occur during times of extreme malnourishment and not during regular and

predictable bouts of fasting (Kempster et al. 2007).

<center>Variability in stable isotope ratios</center>

No significant differences between the stable carbon or nitrogen isotope ratios of males

and females was found in this study. There are no known sex-specific difference in foraging

strategy or location of humpback whales and so similarities in stable isotope ratios are expected.

Previous results also found no significant difference between sexes on feeding grounds (Chapter

2, Witteveen et al. *In review*).

Though there were significant differences observed for $\delta^{13}C$ between sampling regions, it

is arguably more revealing to examine differences between breeding areas when sampling region

is not considered. If $\delta^{13}C$ does reflect origins of feeding when exploring differences between

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sampling regions alone, it could be assumed that humpback whales breeding in Hawaii and Asia

waters forage within the same geographic location and Mexico and Central America animals

forage on distinct grounds as well. However, when breeding areas were the focus of analysis,

similarities between sampling regions were shown to be driven by a relationship between

breeding areas. Thus, if δ^{13}C does serve as an indicator of feeding origins, a complex pattern of

movement between breeding and feeding grounds can be inferred from our results. Similarities in

the ratios of stable carbon isotopes suggest Philippines and Okinawa whales migrate to

isotopically similar feeding grounds, as do Main Mex and Cent Am. The overlap seen between

the remaining breeding areas implies that animals from a given feeding group may not migrate to

any single breeding area. For example, humpback whales belonging to the SEAK group may

migrate to both the Hawaiian Is and Rev Is, resulting in similar carbon ratios for these two

breeding areas. Such movements have been documented previously by photo-identification

analysis (Calambokidis et al. 2001).

Unlike δ^{13}C, δ^{15}N is generally not considered a strong indicator of feeding origins in

marine ecosystems; rather it is used to describe relative trophic position. Since humpback whales

are not foraging on the breeding grounds, differences in stable nitrogen isotope ratios may reflect

differences in trophic position between feeding groups. Following this line of reasoning, our

results would suggest that humpback whales breeding near Cent Am and Main Mex were

foraging at a higher trophic level than those breeding near Ogasawara, for example. However,

several factors prevent such a simple comparison. The unknown time period between cessation

of feeding and sampling on the breeding grounds, as discussed above, is one such factor.

Additionally, the feeding origins of breeding animals must be known. Finally, even if feeding

origins were known, true differences in $\delta^{15}N$ cannot be determined without establishing the $\delta^{15}N$ value at the base of regional food webs (Post 2002).

Assignment of breeding animals to feeding groups

Classification tree results suggest regional patterns of movement between foraging and breeding locations. The western-most breeding grounds are assigned with much greater frequency to the CENT and WEST, which are the western-most feeding groups. Similarly, assignment to COW was most common for the eastern breeding groups in Mexico and Central America. Interestingly, no breeding location showed a strong relationship with either NBC or NGOA. Both feeding groups had some proportion of animals from nearly all breeding areas, however. There are a number of possible explanations for these results. First, these two feeding groups may truly not be dominated by any single breeding area and serve as the feeding grounds for animals from many or all breeding areas. A second, and more likely explanation , is the poor classification of NBC and NGOA in initial classification tree models (Chapter 2, Witteveen et al. *In review*). These two feeding groups exhibited the fewest number of correct classifications on feeding grounds. A weakness in the model to discriminate these groups would easily carry over into the assignment of breeding animals.

Animals were correctly assigned in 56% of cases, which was 3.2 times higher than expected based on random assignment. Perhaps a more meaningful method of determining the success of the classification tree is to compare results with known migratory linkages shown through photo-identification studies. Overall, there is strong consensus between the classification tree results and photo-identification results. In many cases the feeding group that received the majority of tree assignments also received the majority of photographic matches. Assuming that

photo-identification results are relaying an accurate picture of connectivity, than the classification tree model clearly performed better for some areas than for others. The tendency of the model to assign statistically similar breeding areas to different feeding groups suggests that no single parameter is driving the assignments. For example, Okinawa and Philippines showed nearly identical $\delta^{13}C$ means and yet Okinawa was more frequently assigned to WEST and Philippines to CENT. Thus, $\delta^{15}N$ may be more influential for these areas.

There were several breeding areas that showed more diversity in assignments than the others. For example, Hawaiian Is, Baja Pen, and Rev Is, did not show an obvious dominant link to any single feeding group, but showed a range of assignment percentages to all groups. Mean values for these areas tended to be in the low to mid range compared to other areas and were often grouped together in post-hoc tests. It may be that the similarity and relative position of these means hampers the classification tree's ability to assign these breeding areas to a single feeding group. However the diverse classification of these breeding areas may be accurate and reflect substantial mixing of feeding groups at these locations. Support for the mixing of feeding groups can be found in the fact that photographic analysis also reflected diversity in many of the same breeding areas. Together, these results indicate that breeding areas often serve as the migratory destination for several feeding groups (Baker et al. 1986, Calambokidis et al. 1996, Waite et al. 1999, Urbán R et al. 2000, Mizroch et al. 2004).

Overall, these results show considerable promise at assigning breeding humpback whales to their high latitude feeding destinations. While some migratory connections remain nebulous, stable isotope ratios predicted very clear regional patterns of movement and support previous assumptions of the complexity of humpback whale population structure and movement. On its

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own, stable isotope analysis shows considerable strength as a means of exploring facets of

migratory populations and has additional benefits in its low cost and lack of resighting

requirement. When combined with other research methods, stable isotope analysis can further

our understanding of the life history of North Pacific humpback whales.

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Table 3.1. Sample sizes for stable isotope analysis by year for each of the four breeding sampling regions. Also shown are the totals for each breeding area within a region.

Region	Area	Year			
		2004	2005	2006	Total
ASIA	Ogasawara	38	42	23	103
	Okinawa	0	2	20	22
	Philippines	6	6	0	12
	Total	44	50	43	137
HI	Hawaiian Is	124	137	49	310
MEX	Rev Is	17	25	13	55
	Baja Pen	19	0	14	33
	Main Mex	14	0	15	29
	Total	50	25	42	117
CENT AM	Cent Am	9	10	14	33
TOTAL		227	222	148	597

Table 3.2: Mean values (± SE) of $\delta^{13}C$ (‰) and $\delta^{15}N$ (‰) by year and sampling region for breeding North Pacific humpback whales. Letters in the total row indicate similar mean values with respect to year as determined by post-hoc analysis.

	$\delta^{13}C$				$\delta^{15}N$			
Region	2004	2005	2006	Overall Mean	2004	2005	2006	Overall Mean
ASIA	-18.2 ± 0.11	-18.2 ± 0.09	-18.5 ± 0.12	-18.3 ± 0.06	12.0 ± 0.25	12.1 ± 0.22	12.0 ± 0.24	12.1 ± 0.13
HI	-17.8 ± 0.08	-18.2 ± 0.08	-18.0 ± 0.12	-18.0 ± 0.05	13.1 ± 0.14	12.8 ± 0.11	13.0 ± 0.20	13.0 ± 0.08
MEX	-16.8 ± 0.14	-17.5 ± 0.12	-17.5 ± 0.16	-17.2 ± 0.09	13.2 ± 0.18	13.0 ± 0.27	13.7 ± 0.24	13.3 ± 0.13
CENT AM	-16.2 ± 0.19	-16.5 ± 0.15	-16.3 ± 0.14	-16.3 ± 0.09	14.2 ± 0.21	14.9 ± 0.21	15.3 ± 0.19	14.9 ± 0.13
TOTAL	-17.6 ± 0.07 a	-18.1 ± 0.06 b	-17.8 ± 0.08 c	-17.8 ± 0.04	13.0 ± 0.10 a	12.8 ± 0.09 b	13.1 ± 0.14 a	12.9 ± 0.06
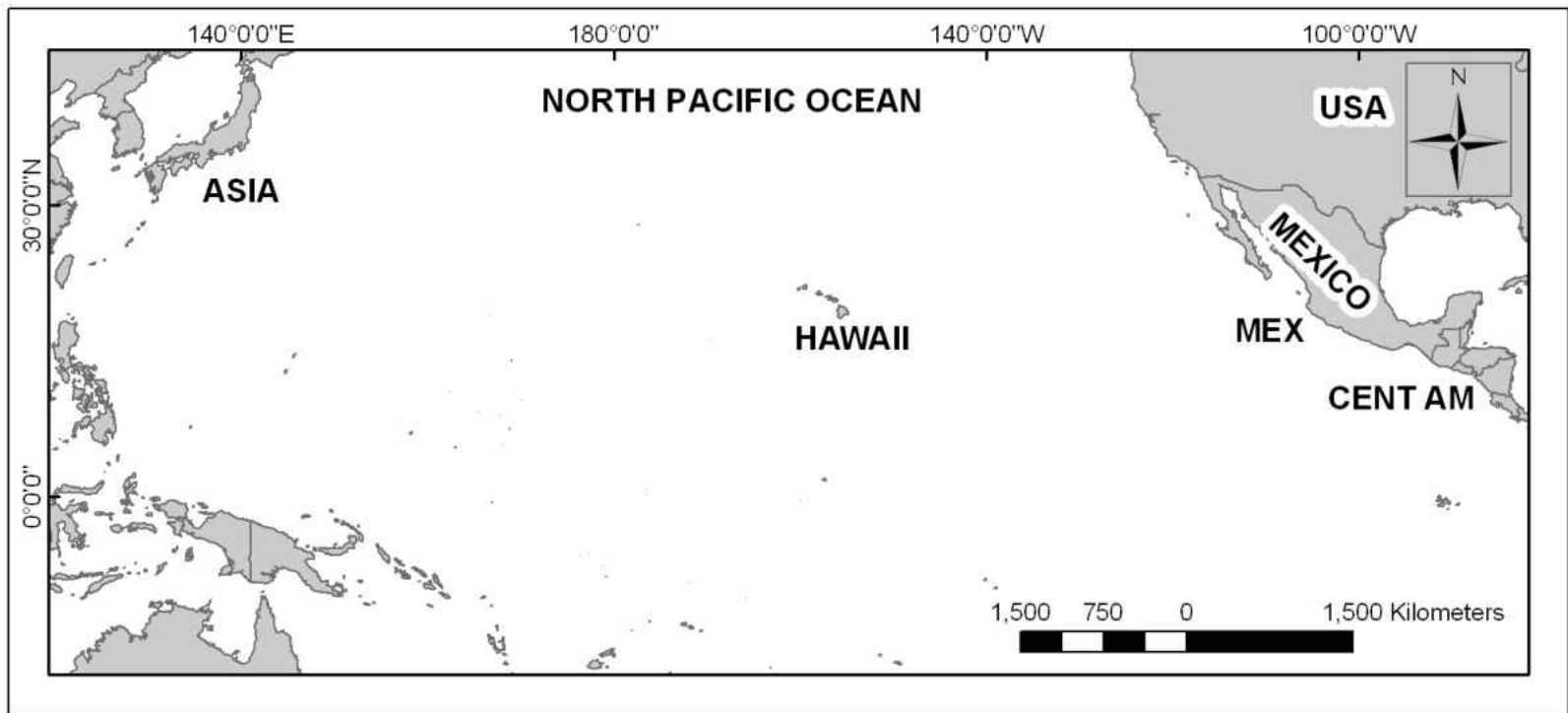
Figure 3.1: Map of the North Pacific Ocean showing the four regions of SPLASH sampling on breeding grounds.
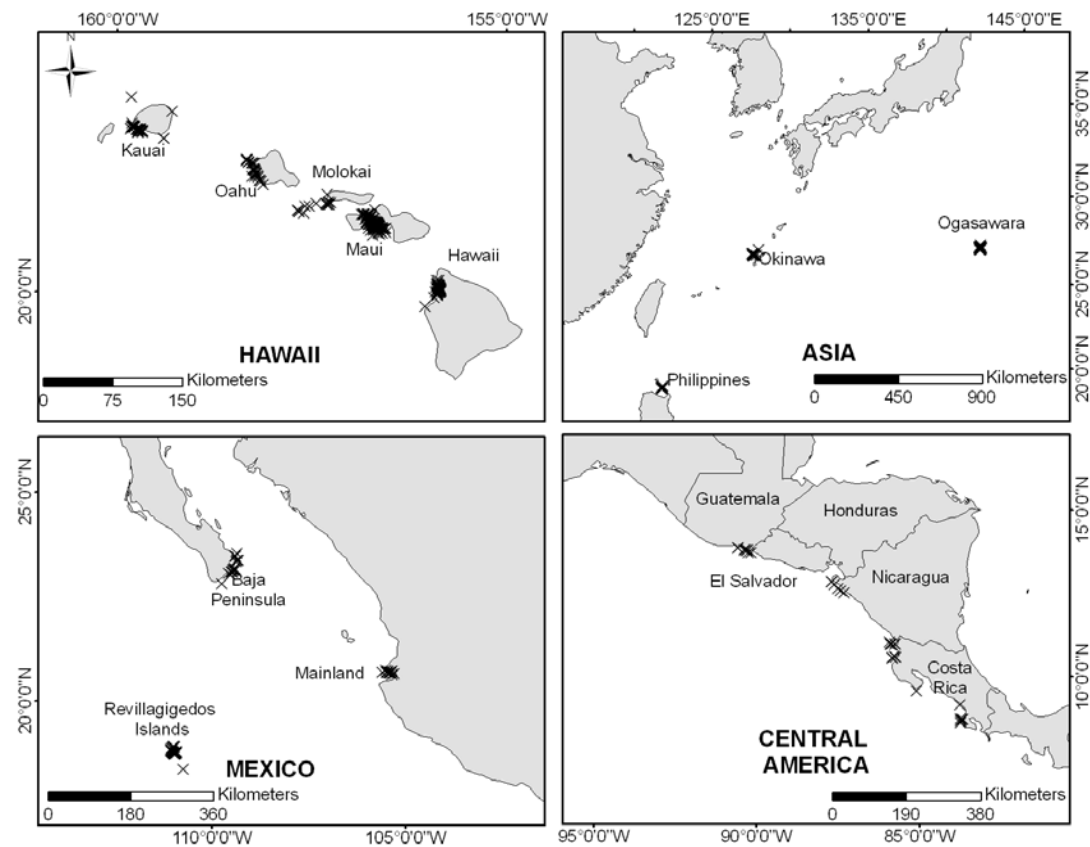
Figure 3.2: Maps of breeding areas within each of the four SPLASH sampling regions. Locations of sample collections (**x**) are also shown.
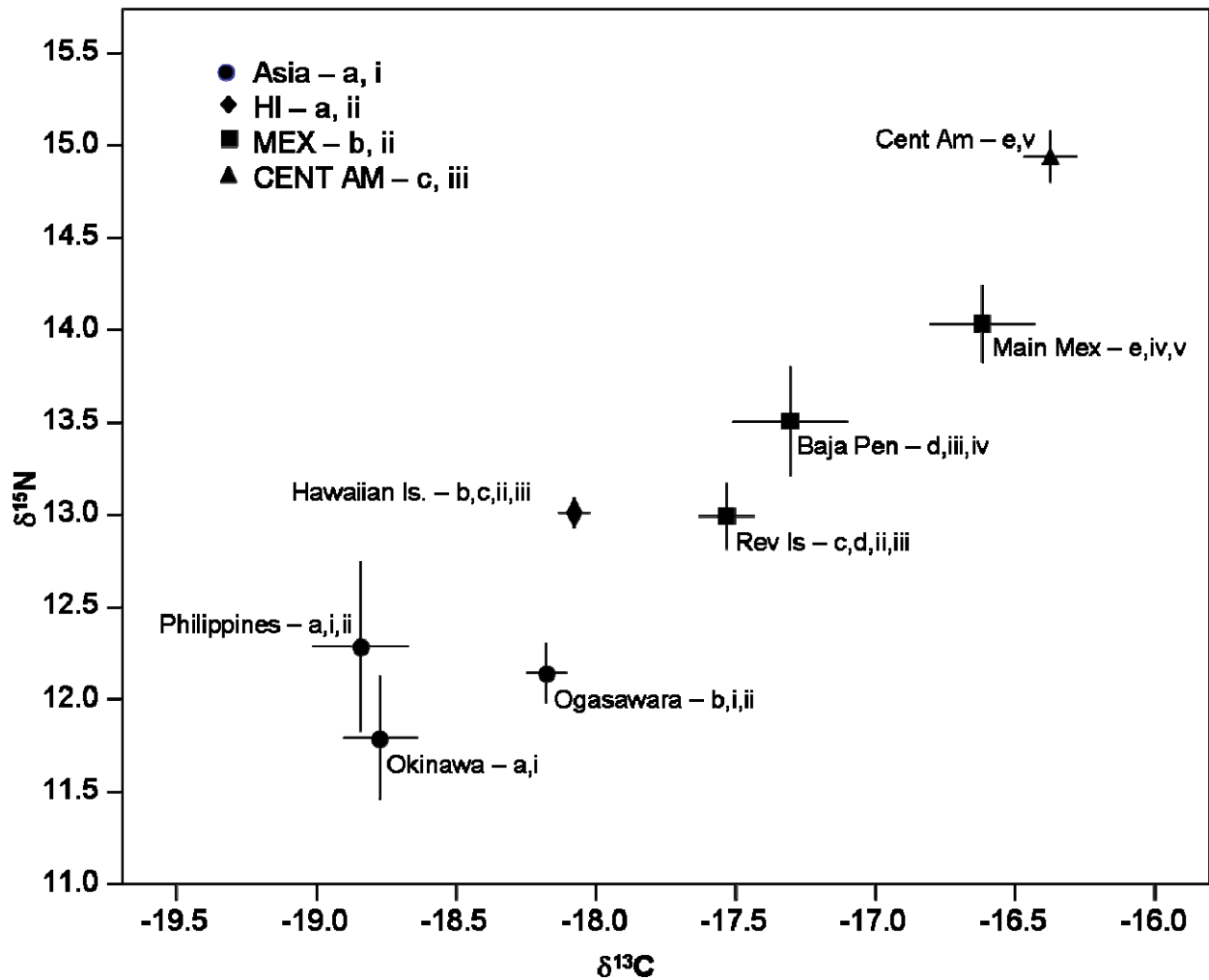
Figure 3.3: Mean values (± SE) of δ^{15}N and δ^{13}C for each of breeding area. Symbols of breeding areas indicate membership to one of four sampling regions. For both sampling regions and breeding areas, letters indicate similar groups with respect to δ^{13}C, while roman numerals indicate similarities with respect to δ^{15}N.
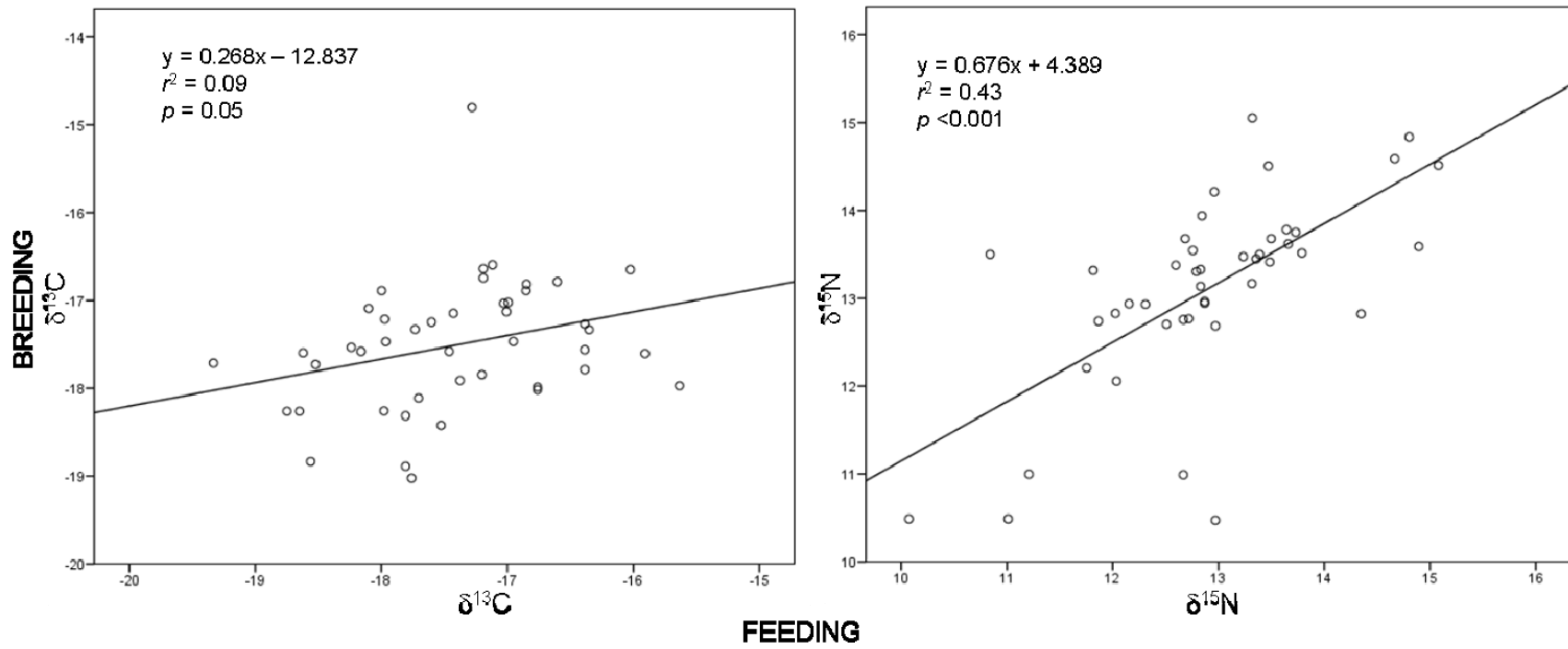
Figure 3.4: Relationships between feeding and breeding values of $\delta^{13}C$ (‰) and $\delta^{15}N$ (‰) for 44 individual humpback whales sampled on both breeding and feeding habitats.
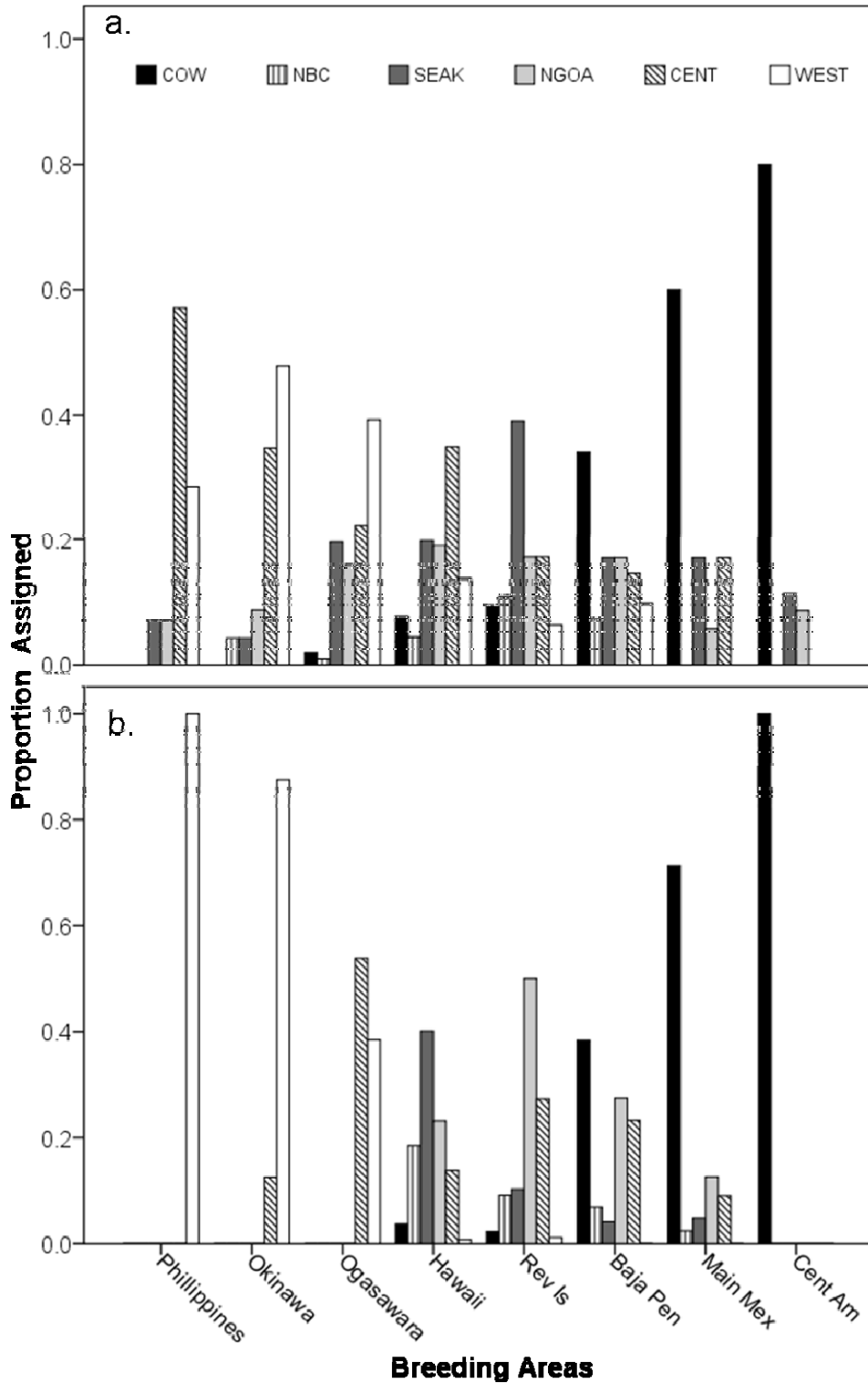
Figure 3.5: Distribution of breeding area animals assigned to feeding groups based on a) classification tree analysis of stable isotope ratios and b) SPLASH photographic matching.

CHAPTER 4: EXPLORATION OF TROPHIC LEVELS OF NORTH PACIFIC HUMPBACK WHALES THROUGH ANALYSIS OF STABLE ISOTOPES: IMPLICATIONS ON PREY SELECTION AND RESOURCE QUALITY

Introduction

Most seasonal or long distance migrations occur in response to seasonal peaks in regional resource availability and, at least with respect to land mammals, are generally characterized by the availability of resources at both ends of the migration (Fryxell 1995, Murray 1995, Corkeron & Connor 1999, Alerstam et al. 2003). Large baleen whales undergo seasonal migrations between high-latitude foraging grounds and low-latitude breeding grounds. In contrast to their land-based relatives, sources of nutrition are not often available on the breeding grounds and many baleen whale species undergo long periods of fasting as a result (Corkeron & Connor 1999). Migration is undoubtedly an energetically expensive behavior in its own right and energy demands likely increase further when coupled with fasting. In addition, activities on the breeding grounds, such as breeding, gestation, and lactation, require an increase in energy demands above standard metabolic requirements (Read 2001). The physical condition of migrant whales when they arrive on their respective breeding grounds is thus critical to survival and reproductive success. Poor body condition of migrants, including baleen whales, has been implicated in declines in reproductive success, changes in offspring sex ratios, delays in migratory timing, and lower annual survival rates (Perrins 1970, Price et al. 1988, Wiley & Clapham 1993, Moller 1994, Stolt & Fransson 1995, Lozano et al. 1996, Sandberg & Moore 1996).

Stores of adipose tissue likely contribute the majority of energy in times of fasting. Migratory birds have been shown to increase fat stores prior to migration by increasing food intake and by selecting diets based, in part, on nutrient content (Pierce & McWilliams 2005). Changes in the fatty acid composition of migratory bird stores is affected by dietary composition, and has direct consequences for the energetic cost of migration (Pierce & McWilliams 2005). It follows that migratory whale species should optimize intake of high quality prey that will contribute most to their fat, or blubber, layer. For marine mammals, the blubber layer serves many functions, including defining hydrodynamic shape, providing buoyancy, insulation from cold water temperatures, and storing energy in the form of lipid (Koopman et al. 2002). As a result, prey choice for baleen whales on their feeding grounds can have significant impacts on future events, including migration, survival, and reproduction.

In the North Pacific, humpback whales (*Megaptera novaeangliae*) migrate from low latitude breeding grounds to geographically distinct feeding aggregations in higher latitudes. Segregation on the feeding grounds has been attributed to the cultural transmission of fidelity to a feeding ground as a result of a calf's early maternal experience (Martin et al. 1984, Baker et al. 1987, Clapham & Mayo 1987).

While on the feeding grounds, humpback whales are classified as generalist in their prey selection and are known to feed on zooplankton, including euphausiids, and small schooling fish, such as Pacific herring (*Clupea pallasii*) and capelin (*Mallotus villosus*). Despite a generalized diet, there are likely significant differences between the specific diets of feeding aggregations, with some groups targeting forage fish and others euphausiids. Location of foraging and prey choice will thus directly impact the variety

and quality of prey available to humpback whales. Humpback whales can lose 1/3 to 1/2 of their body mass while on their breeding grounds because they do little or no feeding (Dawbin 1966, Lockyer 1981b, Baraff et al. 1991, Laerm et al. 1997). During this period of fasting, humpback whales rely almost exclusively on their blubber stores that have accumulated while foraging on the high latitude feeding grounds, while continuing to depend on it for its additional functions (Lockyer 1981b). The quality of prey and its ability to contribute to this energy reserve is therefore critical to survival and reproductive success of humpback whales. As a result, clarifying the number and boundaries of feeding locations can have important implications in management and conservation efforts.

Studying aspects of feeding behavior for humpback whales can be difficult and expensive. Fortunately the analysis of stable carbon and nitrogen isotope ratios has recently emerged as a relatively inexpensive and effective method for exploring trophic position, diet and feeding origins of migratory animals (Hobson 1999). Stable nitrogen isotope ratios become enriched by ~2-5‰ between trophic levels and can, therefore, predict relative trophic position (Minagawa & Wada 1984, Fry 1988, Hobson et al. 1993, 1994, Sydeman et al. 1997, Kurle & Worthy 2002). Previous analysis of stable isotope ratios from humpback whale skin described six isotopically distinct feeding groups and identified likely migratory links between these groups and breeding areas (Chapter 3, Witteveen et al. *In review*). In this study, differences in the relative trophic levels of the North Pacific humpback whale feeding groups were explored through comparison of stable nitrogen isotope ratios of their skin and of primary consumers of regional food webs. How trophic differences among feeding groups may affect their relative success on

breeding grounds are discussed. Findings in this study mark the first attempt to employ stable isotope analysis to infer how differences in regional diets and prey choice may influence aspects of the humpback whale life history.

Methods

Sample collection, preparation, and stable isotope analysis

Humpback whale skin samples were collected for isotopic analysis as part of the Structure of Populations, Levels of Abundance, and Status of Humpback whales (SPLASH) project as described in Witteveen et al. (*In review*). All skin samples were oven dried and lipids were extracted (Chapter 2, Witteveen et al. *In review*). Samples were analyzed for stable carbon and nitrogen isotope ratios using a Finnigan MAT Delta Plus XL isotope ratio mass spectrometer (IRMS). Stable isotope ratios are reported as per mil (‰) using the standard delta (δ) notation according to $\delta X = [(R_{sample}/R_{standard})-1] \times 1000$, where X is ^{15}N or ^{13}C and R is the corresponding ratio of $^{15}N/^{14}N$ or $^{13}C/^{12}C$. Standard reference materials were carbon from Pee Dee Belemnite and atmospheric nitrogen gas. Replicate measurements of internal laboratory standards indicated measurements error of ± 0.10 for both $\delta^{13}C$ and $\delta^{15}N$.

Feeding groups and migratory connections

Previous analysis of $\delta^{13}C$ and $\delta^{15}N$ classified North Pacific humpback whales into six feeding groups (Chapter 2, Witteveen et al. *In review*). These groups were defined as COW (California, Oregon, Washington, and southern British Columbia), NBC (northern British Columbia), SEAK (southeastern Alaska), NGOA (northern Gulf of Alaska),

CENT (western Gulf of Alaska, eastern Aleutian Islands, and Bering Sea), and WEST (western Aleutian Islands and Russia; Figure 4.1). Variables from these groups, including $\delta^{13}C$ and $\delta^{15}N$, were used in classification tree analysis to assign breeding areas to one of these feeding groups, describing migratory connections. Breeding areas were Asia (Philippines, Okinawa and Ogasawara, Japan), the United States (Hawaiian Islands), Mexico (Revillagigedos Islands, Baja Peninsula, and Mainland), and Central America (Figure 4.1; Chapter 3).

Baseline $\delta^{15}N$ of Regional Food Webs

Comparisons of the $\delta^{15}N$ values of humpback whale skin cannot be made without knowledge of the $\delta^{15}N$ values at the base of food webs for each feeding group. Previous studies have used primary consumers, such as copepods (*Calanus sp.*) and filter-feeding bivalves, as good surrogates of food web bases (Kling et al. 1992, Cabana & Rasmussen 1996, Post 2002, Matthews & Mazumder 2005). Thus, in this study, at least one primary consumer from the geographic region of each feeding group, except WEST, was used to set the baseline $\delta^{15}N$ level of regional food webs. With respect to WEST, the $\delta^{15}N$ value obtained for CENT was used in the absence of specific data for that region. Primary consumers used were copepods (Copepoda, *Neocalanus spp., Calanus spp*), weathervane scallops (*Patinopecten caurinus*), mussels (*Mystilus californiana*), and salps (Salpidae) (Table 4.1).

Trophic Ecology

The tropic levels of individual humpback whales were calculated from the following equation:

$$2 + (\delta^{15}N_{specimen} - \delta^{15}N_{primary\ consumer})/2.4$$

where 2 is the trophic position of the primary consumer and 2.4 is the average $\delta^{15}N$ enrichment per trophic level for marine mammals (Hobson 1994, Post 2002). Mean trophic level values for each feeding group were calculated by averaging the trophic levels of individuals within feeding groups. $\delta^{15}N$ of feeding groups were adjusted by the difference between the value of the regional primary producer and the value of the lowest regional primary producer as a means of comparing regional trophic differences based on normalized $\delta^{15}N$.

Statistical analysis

Analyses of variances (ANOVA) were used to explore differences in trophic level between feeding groups. Homogeneous subsets were determined through Tukey's post-hoc tests following analysis. All statistics were conducted within SPSS 15.0 for Windows with a critical value of $\alpha = 0.05$ for all analyses (Moran 2003). Values presented are mean ± SE.

Results

In total, 1105 samples of humpback whale skin from six feeding groups were analyzed for $\delta^{15}N$. Mean values of $\delta^{15}N$ for primary consumers ranged from 8.8 in NGOA to 10.2 in COW; thus primary consumers differed by up to 1.4‰ across feeding groups (Table 4.1).

The overall mean trophic level for North Pacific humpback whales was 3.6 ± 0.02. Feeding groups means ranged from a low of 3.3 ± 0.08 (WEST) to a high of 4.0 ± 0.03 (NGOA) (Figure 4.3). The lowest individual trophic level was 1.4 and was estimated

for an animal sampled in SEAK in 2004. The highest individual trophic level came from

NGOA in 2004 and was estimated at 5.1. δ^{15}N of humpback whale skin increased by an

average of 3.9‰ over primary consumers signifying they were foraging approximate 1.6

trophic levels higher than primary consumers.

Trophic level differed among feeding groups ($F_{5,1099}$= 62.0 $p<0.001$). Post-hoc

tests showed that mean trophic level for NGOA and COW were significantly different

than all other groups. The trophic levels of the remaining four feeding groups did not

differ significantly (Figure 4.2).

Discussion

A mean trophic level of 3.6 for North Pacific humpback whales supports the

assumption that they are generalist predators and likely exploit both fish and zooplankton

species. If the humpback whales sampled in this study were feeding primarily on

zooplankton, it is likely that estimates of trophic level would be closer to those of

cetacean species adhering to a more strict plankton diet, such as the bowhead whale (TL

= 2.8-3.0; Hoekstra et al. 2002). Trophic levels of strict ichthyophagous marine mammals

tend to be higher, such as those estimated for beluga whales (TL = 4.4 - 4.8; Lesage et al.

2001) and ringed seals (TL = 4.4 - 4.6; Hobson et al. 2002; Figure 4.3). Trophic levels

estimated in this study further suggest that humpback whales are feeding at levels similar

to piscivorous pelagic fish, which generally shown at trophic levels between 3 and 4 and

one to two trophic levels above zooplankton (Lesage et al. 2001, Das et al. 2003,

Morissette et al. 2006). Occupying similar trophic levels could indicate the potential for

competition between humpback whales and some fishes. However, such competition

could not be explicitly described without knowledge of the types and abundance of prey each were targeting.

Though COW exhibited the highest mean value of $\delta^{15}N$ (14.7), it had only the second highest trophic level (3.9). The highest trophic level was seen in NGOA (4.0), where average $\delta^{15}N$ was 1.1‰ lower than COW. The discrepancy between $\delta^{15}N$ and trophic levels is due to the substantial difference in the $\delta^{15}N$ values of the primary consumers in each feeding region. While the stable nitrogen isotope ratios of primary consumers (trophic level = 2) were near 9.0‰ for most feeding groups, the COW value was 10.2‰. Failing to account for differences at lower trophic levels and basing estimates of trophic level on $\delta^{15}N$ alone would result in the assumption that COW was feeding at a trophic level considerably higher than all other North Pacific feeding groups. Thus, it is very important to account for differences in the baselines of food webs before making trophic level comparisons (Post 2002).

Species of prey available to humpback whales can vary widely by season and location and, while considered generalists as a species, the trophic levels of feeding groups of humpback whales suggest significant regional differences in the types of prey being targeted. With a trophic level at or near 4.0, it is likely that the diet of the NGOA and COW groups had a diet proportionally higher in fish species than zooplankton, while the remaining groups all had trophic levels closer to 3.5, indicating a more mixed diet of both fish and zooplankton. Field observations provide support for relative trophic level differences. For example, humpback whales have been seen foraging extensively on euphausiid swarms in the eastern Aleutian Islands, an area included in the CENT feeding group with an estimated trophic level of 3.5 (C. Matkin North Gulf Oceanic Society, pers.

comm.). In contrast, the higher trophic level of COW is substantiated by recent observations of a switch from zooplankton to fish for animals feeding off California (J. Calambokidis Cascadia Research, pers.comm). Further, humpback whales foraging near Kodiak Island, Alaska, within the NGOA feeding group, have been shown to target aggregations of capelin (Witteveen et al. 2008)

Such variation in prey use may significantly influence life history parameters of feeding groups. Humpback whales depend on high quality forage to sustain migratory and breeding behaviors through lengthy periods of fasting. Diets of poor quality or quantity may not contribute enough lipid to adipose tissue reserves, which are catabolized during migration and periods of limited nutrient intake (Lockyer 1986, Bairlein 1987, Izhaki & Safriel 1989, Castellini & Rea 1992, Parrish 1997). Lipid content is the primary determinant of energy density, both of which can vary widely across taxa (Anthony et al. 2000). For example, the energy content of euphausiids is relatively low at 0.74 kJ/g (Davis et al. 1998) but over 5 kJ/g for some forage fish (Anthony et al. 2000). Assuming lipid content and energy density are surrogate measures of prey quality, it would follow that humpback whales belong to the COW or NGOA feeding groups may receive more benefits in the form of stored energy from their predation of fish or require smaller quantities of prey than groups foraging on euphausiids, such as WEST or SEAK.

While the benefits of foraging are accrued on feeding grounds, they are realized on breeding grounds and, as such, the impact of foraging location on breeding animals must also be considered. Studies of migratory birds have shown that the quality of resources in one habitat can reduce the reproductive success and productivity at the other habitat (Norris et al. 2004, Hebert & Wassenaar 2005b) and that lipid content of prey is

positively correlated with offspring growth and reproductive success (Forero et al. 2002). Lockyer (2007) reviewed how food energy storage in the form of blubber can be vital to a number of functions, including insulation and reproductive efficiency, in both large migratory and small cetaceans. Further, body condition, food abundance and fertility were all tightly linked in fin whales, a cousin of the humpback whale (Lockyer 1986, 1987a, b, 1990). Analysis of $\delta^{13}C$ and $\delta^{15}N$ showed a strong migration link between Central America and Mainland Mexico and COW (Chapter 3). Assuming prey resource require the same energy to capture, Anthony et al. (2000) states "by selecting for prey quality, in conjunction with maximizing quantity, piscivorous predators can potentially increase their own fitness and the productivity of the population." Thus, based on assumptions regarding energy density and prey quality, animals breeding in these areas should benefit, perhaps in the form of increased survival or fecundity, as a result of higher trophic level feeding within the COW group. Conversely, animals breeding in one of the Asia areas may not incur as many energetic benefits as stable isotope ratios showed that CENT and WEST were their primary foraging locations. Stable isotope models did not assign a dominant breeding area for NGOA foraging animals, which could be a result of a weakness in the model or could indicate that NGOA animals migrate evenly among the breeding groups (Chapter 3). Regardless, benefits resulting from their foraging choices are thus difficult to predict.

As stated previously, humpback whale prey can be highly variable both temporally and spatially, in addition to their energy content. The availability and abundance of prey within the boundaries of each feeding group likely dictates which prey humpback whales actually ingest. If certain prey types are predictably available, it is not

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unreasonable to believe that feeding groups of humpback whales could develop into regional prey specialists. Such specialties would easily become fixed, since segregation of feeding groups has occurred as the result of a cultural transmission of migration routes from mother to calf (Aidley 1981a, Martin et al. 1984, Baker et al. 1987, Clapham & Mayo 1987). Thus, predator selection of a prey resource with relatively low available energy may have significant long term population effects resulting from reductions in body condition and reproductive success (Urton & Hobson 2005, Inger et al. 2006).

There are limitations in this exploration of stable isotope ratios and trophic levels. First of all, discussion of diet composition and trophic position depend on an accurate estimate of stable isotope enrichment of ^{15}N between humpback whales and their prey. Unfortunately, there are presently no published trophic enrichment factors for humpback whales. Other studies have used enrichment factors ranging between 2.4 to 3.8‰ (Hobson & Welch 1992, Hoekstra et al. 2002, Born et al. 2003, Das et al. 2003). We used the lowest value of 2.4‰ because it has been applied to previous studies of marine mammals, including cetaceans (Hobson et al. 1996, Das et al. 2003). Choosing a higher trophic enrichment factor would decrease our estimates of trophic level, changing our assumption of a fish-based diet for COW and NGOA to a mixed diet and a mixed diet to a zooplankton-dominated diet for the remaining feeding groups. However, despite these changes, the relative differences and conclusions about differences in prey types between feeding groups would remain the same.

Calculations of trophic level also depend highly on the turnover rate of assimilated tissues if diets are not constant throughout the feeding season. The turnover rates of tissues are proportional to their metabolism, with active tissues (i.e., skin or

muscle) showing faster turnover than inert tissues (i.e., baleen or bone) (Tieszen et al. 1983, Schell et al. 1989a, b, Hobson & Clark 1992a, MacAvoy et al. 2006, Podlesak & McWilliams 2006). Though never empirically tested, the skin of rorqual whales likely exhibits high metabolic rates and a turnover rate of 7-14 days for humpback whale skin has been suggested (Todd 1997). Thus, estimates here may reflect the trophic level of only the past two weeks to one month of foraging.

More information is needed to elucidate how prey use may be influencing life history factors such as reproductive success. First, more specific diet composition for each feeding group needs to be described. Fortunately, with the recent advancements in stable isotope mixing equations, feeding group diets could be modeled if a variety of prey resources from each region were available for analysis (Phillips & Gregg 2001, 2003, Newsome et al. 2004, Phillips et al. 2005). Dietary mixing models in this manner would allow for more specific diet comparisons to be made, rather than comparing generalized fish versus zooplankton diets. If data on life history parameters, including but not limited to, calf and adult survival, fecundity, and body condition, were available, correlations between these parameters and dietary differences could be explored. Fortunately, with the growing number of long-term datasets for regional humpback whale populations and the recent efforts of SPLASH, some parameters may be obtainable.

This study represents the first exploration into trophic level differences among humpback whales foraging in the North Pacific. Analysis of stable carbon and nitrogen isotope ratios has shown that there may be significant differences in the prey being utilized between feeding groups. These results highlight the need for additional research

focused on diet composition within each feeding group, as previous studies have shown

that prey choice and diet can have significant impacts on fitness.

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Table 4.1: Mean (± SE) stable nitrogen isotope ratios (‰) and sample sizes for humpback whales and primary consumers for each of the distinct feeding groups of humpback whales in the North Pacific (Witteveen et al. *In review*). Also shown are the trophic levels (TL) of humpback whales for each group.

	Humpback Whales			1° Consumers			
Group	*n*	$\delta^{15}N$	TL	*n*	$\delta^{15}N$	Species	Sources
WEST	81	12.3 ± 0.19	3.3 ± 0.08		9.1*	-	-
CENT	282	12.6 ± 0.07	3.5 ± 0.03	57	9.1 ± 0.13	*Neocalanus spp., Calanus spp., Patinopecten caurinus*	Hirons (2001), Andrews unpubl. data
NGOA	199	13.6 ± 0.07	4.0 ± 0.03	86	8.8 ± 0.04	*Calanus spp., Patinopecten caurinus*	Hirons (2001), Andrews unpubl. data
SEAK	227	12.7 ± 0.06	3.4 ± 0.03	10	9.3 ± 0.10	*Patinopecten caurinus*	Andrews unpubl. data
NBC	135	13.0 ± 0.08	3.5 ± 0.03	42	9.4 ± 0.09	*Mystilus californiana*	Markel unpubl. data
COW	181	14.7 ± 0.07	3.9 ± 0.03	15	10.2 ± 0.69	*Salpidae, Copepoda*	Miller (2006), CSCAPE 2006
Total	1105	13.2 ± 0.04	3.6 ± 0.02	210			

* No data from primary consumers in the WEST feeding group were available. The value shown is from the CENT.

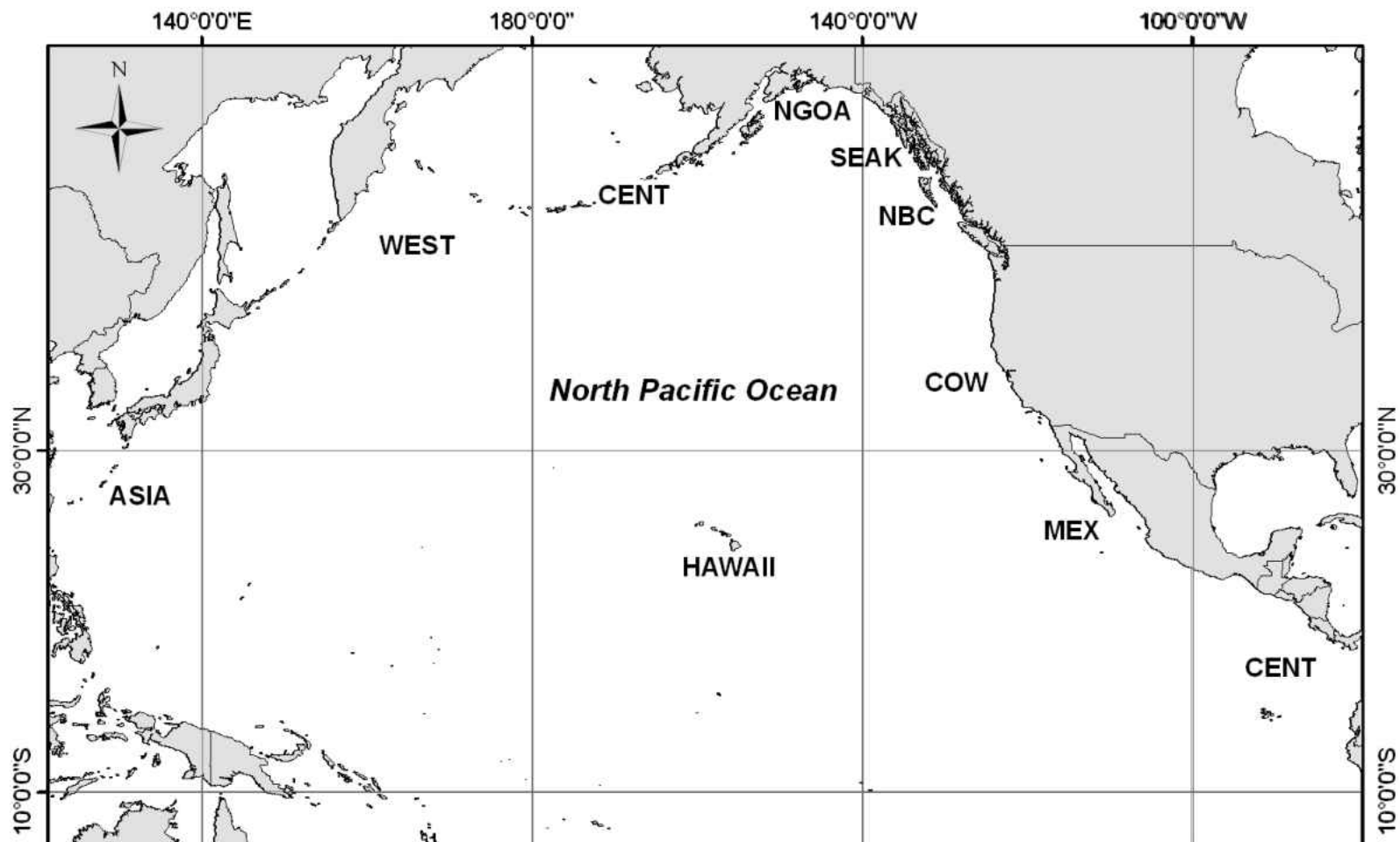Figure 4.1: Map of the North Pacific showing breeding and feeding locations of SPLASH sample collection.
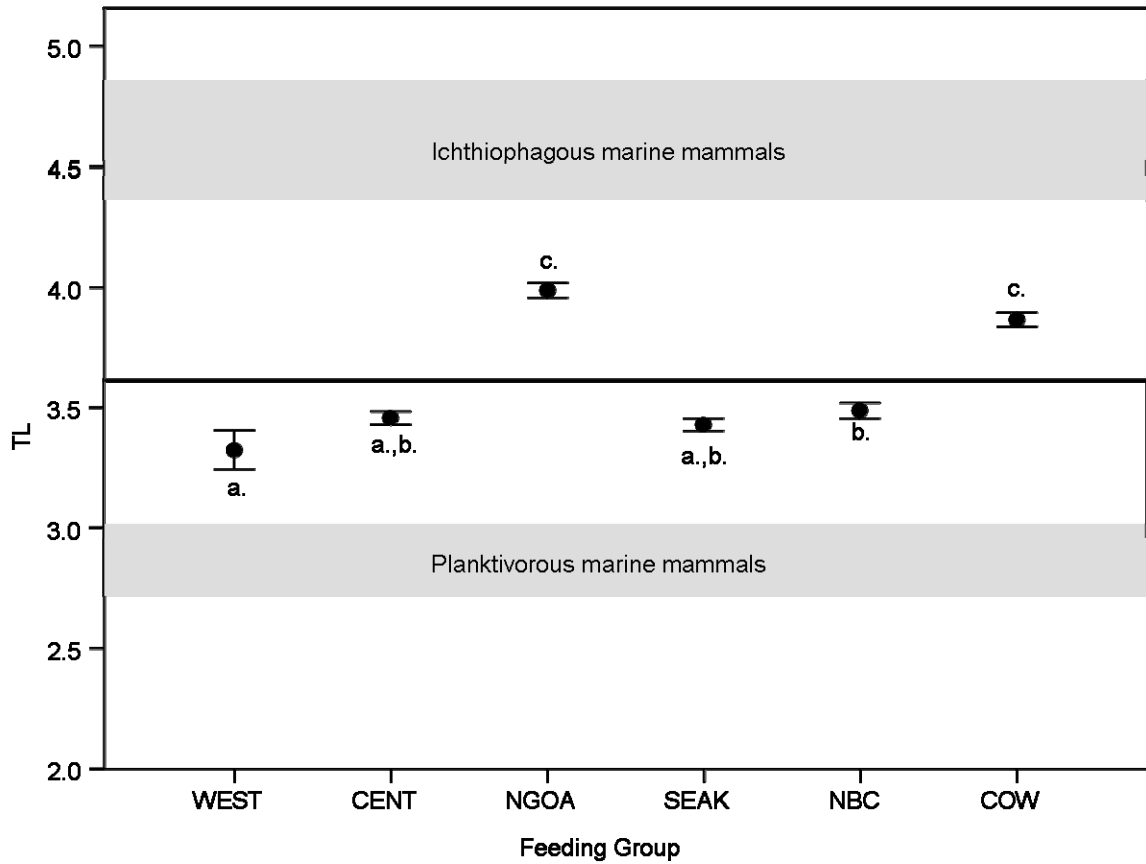
Figure 4.2: Mean (± S.E.) TL for each of the six feeding groups of North Pacific humpback whales. The solid black line represents the overall mean values for all groups. Shaded regions represent the range in trophic levels for strictly fish eating (4.4 to 4.8) and strictly plankton eating (2.8 to 3.0) marine mammals.

CHAPTER 5: MODELING THE DIET OF HUMPBACK WHALES: A CASE STUDY USING STABLE CARBON AND NITROGEN ISOTOPES

Introduction

The North Pacific humpback whale (*Megaptera novaeangliae*) is a marine

predator with a life history highlighted by an extensive seasonal migration. In general,

these whales spend the winter months in warmer, low-latitude waters where they breed

and give birth before migrating to higher latitudes waters to forage. Because humpback

whales fast during migration and while on breeding grounds, they are exposed to periods

of nutritional stress and potential reductions in body condition (Dawbin 1966, Lockyer &

Brown 1981, Baraff et al. 1991, Laerm et al. 1997). Thus, food quality and intake need to

be optimized on the feeding grounds in order to sustain migration and breeding behavior

and, for females, lactation and pregnancy (Read 2001, Craig et al. 2003).

On North Pacific feeding grounds, humpback whales are classified as top-level

predators and are known to consume substantial amounts of prey. These whales are

considered generalists in their prey selection, feeding seasonally on zooplankton, such as

krill (*Thysanoessa* spp. and *Euphausia pacifica*), and pelagic schooling fish, including

capelin (*Mallotus villosus*), Pacific herring (*Clupea pallasii*), and juvenile walleye

pollock (*Theragra chalcogramma*) (Nemoto 1959, Krieger & Wing 1984, 1986).

However, past observations and analyses suggest differences in trophic level and prey

use among feeding groups (Chapter 4, Witteveen et al. *In review*). Variation in prey use

may result in inconsistent pressures on prey populations and result in differences in body

condition between feeding aggregations. Examining consumption by humpback whales

therefore contributes valuable information about complex ecosystem linkages and

predator-prey dynamics and may provide insight into differences in population parameters of regional feeding groups.

Unfortunately, studying the foraging habitats and prey preferences of whales can be very difficult. Identifying prey in cetacean diets with certainty requires analysis of the stomach contents of harvested or beached whales (Thompson 1940, Klumov 1963) or direct observation of prey in the mouths of surface-feeding animals. However, both means of exploring diet composition are infrequent and can bias results. Fecal samples from free-swimming whales may also provide dietary insights, but their collection is rare.

Fortunately, analyses of stable carbon and nitrogen isotope ratios are increasingly being used as a technique for exploring trophic position, diet, and feeding origins of migratory animals (Hobson 1999). The stable carbon and nitrogen isotope ratios in animal tissues reflect that of their assimilated diet (Deniro & Epstein 1978, 1981, Rau et al. 1983, Wada et al. 1987, Fry 1988). Thus, stable isotope ratios are often used to explore dietary inputs (Phillips & Gregg 2001, Phillips & Eldridge 2006). Distinct isotopic signatures of both predator and prey can be used in mass balance equations (mixing models) to determine the relative contribution of a variety of prey sources in the predator's diet (Phillips & Gregg 2003, Phillips et al. 2005).

In this study, ratios of stable carbon and nitrogen isotopes were used to explore the foraging ecology of humpback whales of the Kodiak archipelago, which represents one feeding aggregation of humpback whales within the North Pacific (Chapter 2, Waite et al. 1999, Witteveen et al. 2006, Witteveen et al. 2008, *In review*). A dietary mixing model was used to predict the composition of potential humpback whale diets using carbon and nitrogen isotope ratios of humpback whale skin and regional prey sources.

Methodologies used here may be applied to other feeding aggregations of humpback whales and more specific comparisons of regional diets can be made. Following such comparisons, it may be possible to determine how differences in regional diets and prey choice may influence humpback whale population parameters. Overall, this study shows the utility of stable isotope analysis in exploring areas of cetacean ecology that are often difficult to study.

Methods

Study area and period

The study area encompassed the waters of the eastern Kodiak archipelago (Figure 5.1). Humpback whale samples were collected June –August in 2004 - 2006 in two sampling regions; North and South. Prey samples were collected in May and August of 2003 and 2004 and August only in 2005.

Sample collection

Samples for stable isotope analysis were collected from free-ranging humpback whales using a hollow-tipped biopsy dart fired by a modified .22 rifle. Skin that was sloughed following acrobatic displays (such as breaching and tail slapping) was also collected for analysis. At each sampling event, the date, location (latitude and longitude), group composition, and general whale behavior were recorded. In addition, identification photographs of tail flukes of sampled animals were collected whenever possible. As soon as possible after collection, samples were preserved by freezing.

Fish were collected for stable isotope analysis during mid-water trawl and hydroacoustic surveys conducted within the study area between 2003 and 2005 (Figure

5.1). Multiple passes with a commercial mid-water trawl net with a 22-mm mesh cod-end liner were made through acoustic scattering layers to ensure representative sampling. Species composition, species counts, and fish size were determined for each tow. Species caught in tows that were considered potential humpback whale prey were schooling fishes measuring less than 30 cm and included capelin, eulachon (*Thaleichthys pacificus*), juvenile walleye pollock, and Pacific herring (Nemoto 1959). Isotopic values for euphausiids (*Thysanoessa spinifera*) and Pacific sandlance (*Ammodytes hexapterus*) were used from samples originally collected within the study area for a separate stable isotope study (Williams 2008).

<center>Sample preparation and stable isotope analysis</center>

Samples were prepared for stable isotope analysis through a multi-step process that included oven drying, extraction of lipids, and homogenization (Witteveen et al. *In review*). Samples were analyzed for stable carbon and nitrogen isotope ratios using a Finnigan MAT Delta Plus XL isotope ratio mass spectrometer (IRMS) at the University of Georgia Institute of Ecology Stable Isotope Laboratory.

Stable isotope ratios were 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 $^{13}C/^{12}C$. Standard reference materials were carbon from Pee Dee Belemnite and atmospheric nitrogen gas.

Analytical errors were ± 0.1 for both $\delta^{13}C$ and $\delta^{15}N$ (Chapter 2, Witteveen et al. *In review*).

Statistical analysis

Sex was determined for a subset of sampled animals ($n = 20$) through genetic analysis (SPLASH unpubl. data). A preliminary analysis of variance (ANOVA) controlling for year determined sexes did not differ. Results of ANOVA were not significant for $\delta^{13}C$ ($F_{2,16} = 0.07$, $p = 0.791$) or $\delta^{15}N$ ($F_{2,16} = 1.22$, $p = 0.285$). Therefore, samples of known and unknown sex were pooled for the remainder of the analyses and sex was removed as a variable in subsequent analyses.

For humpback whale samples, influences of distance from shore, month, latitude and longitude of the sampling location on $\delta^{13}C$ and $\delta^{15}N$ were explored through simple linear regression. Distance from shore (in km) was calculated based on the distance from the sample location to the nearest coastline. Differences in mean stable isotope ratios were tested using ANOVA. $\delta^{13}C$ and $\delta^{15}N$ were tested separately with year and sampling region as factors. Homogeneous subsets (Tukey's post-hoc) were used to determine if any years or sampling regions should be combined to form sampling groups.

Sources of variability in the stable isotope ratios of collected humpback whale prey were also explored by species through ANOVA. Factors explored were size class and year of sample collection. Size class was based on frequency distribution of measured lengths (cm). Mean values of $\delta^{15}N$ and $\delta^{13}C$ were then tested for differences between prey groups using ANOVA. Prey not shown to be significantly different through homogenous subsets were grouped into prey categories for input into diet modeling (Phillips et al. 2005).

All statistics were conducted within SPSS 15.0 for Windows with a critical value of $\alpha = 0.05$ for all analyses (Moran 2003). Values presented are mean ± SE.

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Diet Modeling

Isotope mixing models can be used to explore the relative contribution of prey sources to a consumer's diet (Phillips & Gregg 2003). In standard mixing models, the number of elements (n) used will allow for the contribution of $n+1$ sources to be evaluated in a mixture. This limitation can be problematic for generalist predator, such as the humpback whale. The program IsoSource (http://www.epa.gov/wed/pages/models.htm) solves this problem by using an iterative approach to produce all feasible source combinations based on isotope values for any number of sources (Phillips & Gregg 2003). The model requires the user to adjust isotope values of the consumer with respect to appropriate discrimination factors. A discrimination factor (Δ) is equal to the difference in δX between the consumer and its prey (Montoya 2007). Additionally, the mass balance tolerance (in ‰) permitted about the mean of the consumer's tissues and an interval increment must be specified (Phillips & Gregg 2003, Newsome et al. 2004, Urton & Hobson 2005). To date, there are few published discrimination factors of carbon and nitrogen for marine mammals in general and no published results for cetaceans. However, Hobson (1996) reported factors of ~1.3‰ for $\Delta \delta^{13}C$ and ~2.4‰ for $\Delta \delta^{15}N$ for captive harp seals, which have subsequently been used as factors for cetaceans (Todd 1997, Lesage et al. 2001, Das et al. 2004, Hammill et al. 2005). Therefore, these values were used for humpback whale stable isotope ratios in this analysis, using source increments of 1%. Mass balance tolerance was initially set at 0.1‰ based on analytical measurement error. On occasions where this tolerance level produced zero feasible solutions, tolerance was increased to 0.2‰ and the analysis was rerun (Phillips & Gregg 2003). Potential diets of humpback whales were

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estimated for all samples combined. Diets were then remodeled with prey showing the smallest contribution to the initial diet removed. This simpler diet was then used to model the diets of each of the humpback whale diets of each sampling group separately. Feasible solutions for the distributions of each prey item to the humpback whale diets are presented as means followed by 25th to 75th percentile ranges. Presenting a single proportion, such as mean, is discouraged as it can often misrepresent the uniqueness of the results (Phillips & Gregg 2003, Urton & Hobson 2005).

Since discrimination factors for humpback whales were not explicitly known, a sensitivity analysis of these factors on diet results was conducted. The model for all samples was reexamined with $\Delta \delta^{13}C$ values of 0 and 1 and $\Delta \delta^{15}N$ values of 0, 2, and 4 and results compared with the base model ($\Delta \delta^{13}C = 1.3$, $\Delta \delta^{15}N = 2.4$).

Results

Sampling results

Between 2004 and 2006, 96 samples were collected from humpback whales within the study area. Sample sizes for each of the three years beginning in 2004 were 29, 45, and 22 respectively, with 42 samples collected in the North and 54 in the South (Table 5.1, Figure 5.1). Sampling effort was relatively even across years and the increase in sample size in 2005 was due primarily to an increase in the number of animals within the study area. A total of 116 samples from four fish species were collected for stable isotope analysis as humpback whale prey within the study area (Figure 5.1): Capelin had the most samples ($n = 51$), followed by eulachon ($n = 39$), Pacific herring ($n = 15$), and juvenile walleye pollock ($n = 11$).

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<u>Humpback whales</u>

The mean value of $\delta^{13}C$ for humpback whale skin was -17.6 ($\pm$ 0.06) for all years combined and ranged from -17.3 ($\pm$ 0.09) in 2004 to -18.2 ($\pm$0.10) for 2006 (Table 5.1). The mean value of $\delta^{15}N$ was 13.4 ($\pm$ 0.09) for all years combined with a maximum mean value of 13.5 ($\pm$ 0.10) in 2004 and a minimum of 13.0 ($\pm$ 0.14) in 2006 (Table 5.1). $\delta^{13}C$ from humpback whales sampled in the North (-17.7 $\pm$ 0.08) sampling region were less depleted then those from the South (-17.9 $\pm$ 0.06; Table 5.1). A mean $\delta^{15}N$ value of 13.7 ($\pm$0.13) for North samples was also more enriched than the mean of 13.1 ($\pm$0.09) from the South.

Neither latitude ($F_{1,94} = 0.15$, $r^2 = 0.002$, $p = 0.703$) nor longitude ($F_{1,94} = 0.0$, $r^2 = 0.00$, $p = 0.996$) affected $\delta^{13}C$ values in humpback whale skin. The month in which samples were collected was also not significant for $\delta^{13}C$ ($F_{1,94} = 0.59$, $r^2 = 0.01$, $p = 0.446$). However, $\delta^{13}C$ decreased with increasing distance from shore, ($F_{1,94} = 10.42$, $r^2 = 0.10$, $p = 0.002$).

As with stable carbon isotope ratios, no significant relationships between Kodiak Island humpback whale skin $\delta^{15}N$ and either latitude ($F_{1,94} = 0.00$, $r^2 = 0.00$, $p = 0.967$) or longitude ($F_{1,94} = 2.97$, $r^2 = 0.03$, $p = 0.088$) were found. Also similar to $\delta^{13}C$ was a significant decrease in $\delta^{15}N$ as distance from shore increased ($F_{1,94} = 17.40$, $r^2 = 0.16$, $p<0.001$). In contrast, however, $\delta^{15}N$ decreased linearly with increasing month of sample collection ($F_{1,94} = 12.95$, $r^2 = 0.12$, $p = 0.001$).

Mean values of $\delta^{13}C$ in humpback whale skin differed significantly between years (ANOVA, $F_{2,90} = 26.43$, $p<0.001$) and regions ($F_{1,90} = 4.87$, $p = 0.03$), but the interaction

between the two was not significant ($F_{2,90} = 1.73$, $p = 0.183$). Post-hoc tests showed that $\delta^{13}C$ values for humpback whales in 2004 were significantly different that those in 2005 and 2006 (Figure 5.2). Mean values of $\delta^{15}N$ did not vary significant between years ($F_{2,90} = 1.72$, $p = 0.184$), but did between regions ($F_{1,90} = 13.44$, $p < 0.001$). As with $\delta^{13}C$, the interaction between years and regions was also not significant for stable nitrogen isotope ratios ($F_{2,90} = 1.18$, $p = 0.313$).

Humpback whale samples were pooled into groups based on similarities in mean stable isotope values for both sampling regions and year. Samples collected in 2005 and 2006 were pooled together, but remained separate from 2004, since mean $\delta^{13}C$ from these years did not differ. Sampling regions were significantly different with respect to both stable isotope ratios, so were also separated. Thus, four sampling groups were formed and used in diet modeling. They were 2004 samples from the North (04N), 2004 samples from the South (04S), 2005 and 2006 samples from the North (0506N) and finally 2005 and 2006 samples from the South (0506S; Figure 5.2).

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Humpback whale prey
Mean values of $\delta^{13}C$ of humpback whale prey species varied from a high of -17.5 (± 0.14) for eulachon to a low of -19.7 (± 0.04) for adult euphausiids. Adult euphausiids also had the lowest mean value for $\delta^{15}N$ (10.7 ± 0.11), while herring had the highest (13.5 ± 0.16).

A bimodal distribution of lengths was apparent for capelin, eulachon, and walleye pollock (Figure 5.3). Thus two sizes classes (small and large) for these species were established (Table 5.2). Year and age class could not be tested together for walleye

pollock since all of the small pollock were collected in 2004 and all of the large pollock were collected in 2005. Thus, size class was the only factor explored and was found to differ for both $\delta^{13}C$ ($F_{1,9} = 60.22$, $p < 0.001$) and $\delta^{15}N$ ($F_{1,9} = 97.27$, $p < 0.001$). For capelin, mean values $\delta^{13}C$ were significantly influenced by size class ($F_{1,46} = 31.61$, $p < 0.001$), but not year ($F_{2,46} = 0.72$, $p = 0.494$) nor the interaction between the year and size class ($F_{1,49} = 0.23$, $p = 0.637$). Similar results were seen for the stable carbon ratios of eulachon ($F_{1,34} = 4.32$, $p = 0.045$ for age class, $F_{2,34} = 0.19$, $p = 0.827$ for year, and $F_{1,34} = 2.03$, $p = 0.163$ for the interaction). With respect to $\delta^{15}N$, year ($F_{1,46} = 0.04$, $p = 0.958$), size class ($F_{2,46} = 2.07$, $p = 0.157$), and the interaction ($F_{1,46} = 2.07$, $p = 0.157$) were not significant for capelin. These factors failed to show significance for eulachon as well ($F_{2,34} = 1.95$, $p = 0.158$ for year, $F_{1,34} = 0.32$, $p = 0.578$ for size class, and $F_{1,34} = 0.00$, $p = 0.969$ for the interaction). The variability of the stable isotope ratios of herring were not explored due to the fact that only one size class from one year was collected.

ANOVA indicated that, when applicable, species should be separated into size classes but not separated by year, resulting in seven categories of collected prey; small and large capelin, small and large eulachon, small and large walleye pollock, and Pacific herring (Figure 5.4). Mean values of $\delta^{13}C$ were significantly different for these categories ($F_{6,109} = 27.78$, $p < 0.001$). Post-hoc tests revealed small capelin were significant different than all others, with the remaining categories distributed among three additional subsets. Mean values of $\delta^{15}N$ were also significantly different ($F_{6,109} = 84.87$, $p < 0.001$), but post-hoc tests produced only two homogenous subsets (Figure 5.4). One subset contained small walleye pollock and large and small capelin, while the other contained herring, both size classes of eulachon and large walleye pollock. Based on

these subsets, large capelin and small walleye pollock were combined into a single category as were large eulachon and herring, while all others remained as independent prey categories (Table 5.2).

Diet Modeling

Stable isotope ratios of local humpback whales and potential prey were used in Program IsoSource to model possible contributions of each prey species to the humpback whale diet for all samples combined and then for each sampling group separately (Figure 5.5).

When all samples were combined, Kodiak Island humpback whales were found to rely significantly on euphausiids (67%, 63-71%), Pacific sandlance (13%, 5-19%), and the small pollock and large capelin group (12%, 5-18%). Small capelin (3%, 1-4%) and large pollock (3%, 1-4%) contributed to a lesser extent, while small eulachon (2%, 0-4%) and the large eulachon and herring group (1%, 0-1%) showed only minor contributions to the diet (Figure 5.6a).

As a result of their low contribution, large pollock, small capelin, small eulachon and the large eulachon and herring group were removed from subsequent diet models. Also, large capelin and small pollock were separated and entered as separated prey sources. This separation was done in response to questions regarding consumption of capelin versus walleye pollock (Witteveen et al. 2006, Witteveen et al. 2008).

The removal of minor prey contributors resulted in a decrease in the range of feasible contributions of euphausiids (60%, 57-63%), while increasing the range of proportions for Pacific sandlance (14%, 5-21%), capelin (12%, 5-19%) and walleye

pollock (14%, 6-20%) for all humpback whale skin samples combined (Figure 5.6b).

Applying the simplified, four input diet model to each of the humpback whale sampling

groups suggested that diets were move diverse in the North, while euphausiids dominate

diets in the South (Figure 5.6b). Humpback whales in the 04N sampling group showed

the most uniform distribution among prey categories with euphausiids (23%, 20-26%),

large capelin (21%, 8-31%), Pacific sandlance (25%, 11-38%) and small pollock (31%,

19-42%) each feasibly contributing at or near 25%. Feasible diets for 04S were

dominated by euphausiids (34%, 33-34%) and small pollock (64%, 63-65%), with only

slight occurrences of large capelin (1%, 0-2%) and Pacific sandlance (1%, 0-2%; Figure

5.6b). Diets in both regions during 2005 and 2006 were quite different than the previous

year's model. For 0506N, the contribution of euphausiids (48%, 48-48%) and large

capelin (51%, 50-52%) were almost identical, while euphausiids (94%, 93-96%) alone

were substantially more dominant in the South (Figure 5.6b).

Sensitivity to discrimination factors

A total of seven additional IsoSource models were run to explore the influence of

discrimination factors on feasible diet inputs. Only two of these models were able to

produce results. In the first successful model $\Delta \delta^{13}C$ was set to 1‰ while $\Delta \delta^{15}N$

remained at the original value of 2.4‰. In the second, $\Delta \delta^{13}C$ remained at the base value

of 1.3‰ while $\Delta \delta^{15}N$ was decreased to 2‰. Decreasing $\Delta \delta^{13}C$ led to an increase in the

mean estimated contributions of euphausiids (43% from 60%) and an increase in the

contribution of small pollock (37% from 15%, Table 5.3). A decrease in $\Delta \delta^{15}N$ of 0.4‰

also led to a decrease in the contribution of euphausiids (45% from 60%), but led to an

increase in the large capelin (39% from 12%) contribution rather than small pollock (Table 5.3).

Discussion

Variability in δ^{13}C and δ^{15}N

δ^{13}C values of humpback whale skin decreased with distance from shore of sample collection. Stable carbon isotopes ratios are known to exhibit a gradient with respect to distance, with values becoming increasingly depleted with increasing distance from shore (McConnaughey & McRoy 1979, Hobson 1993, Burton & Koch 1999).

In addition to this near shore to offshore pattern of depletion, δ^{13}C ratios have also been shown to vary with latitude and longitude (Rau et al. 1982, Dunton et al. 1989, Goericke & Fry 1994, Kelly 2000). The fact that stable carbon isotope ratios did not change with either latitude or longitude in this study was expected because of the relatively small size of the study area; sampling locations varied by less than two degrees for both latitude and longitude. δ^{13}C is frequently used to distinguish origins of feeding. Therefore results from this study support the hypothesis that the Kodiak Archipelago represents a single feeding destination for North Pacific humpback whales (Waite et al. 1999, Witteveen et al. 2004, 2006, 2007).

Also expected was the lack of significant variation in δ^{13}C with respect to month of sample collection. The isotopic signature of carbon at the base of the food chain is established at the start of the season and persists throughout, with only minor changes resulting from trophic enrichment and internal fractionation in longer-lived consumers (Saupe et al. 1989). In contrast, δ^{15}N was negatively related to month of sample. Higher

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δ^{15}N in earlier months may be an artifact of nutritional stress caused by fasting, which has been shown to increase enrichment of ^{15}N as the result of nitrogen recycling (Cherel et al. 2005). In other words, fasting animals are essentially feeding on themselves and should appear to be feeding higher trophically than expected (Hobson et al. 1993, Gannes et al. 1998, Oelbermann & Scheu 2002, Cherel et al. 2005). As a migratory and fasting species, humpback whales should exhibit higher δ^{15}N earlier in the feeding season as these animals arrive on the feeding grounds following weeks or months of fasting. As the season progresses, δ^{15}N should decrease as they fall into equilibrium with the whale's diet and trophic level.

Pair-wise comparisons revealed that mean values of δ^{13}C were significantly different in 2004 than in 2005 and 2006 and the δ^{15}N values were significantly different between the North and South sampling regions. Differences in stable carbon isotope ratios between years may be due to interannual changes in the carbon base of the food web. This possibility could be tested with further analysis of prey resources feeding at various trophic levels. Though some prey resources were explored in this study, samples from different years were pooled and, unfortunately, no prey samples were available for 2006. Differences in δ^{15}N between sampling regions were not likely an artifact of geographic patterns in stable isotope ratios as no significant relationships were seen between δ^{15}N and either latitude or longitude. It is more likely that differences were the result of prey being consumed, with animals in the South groups feeding at a lower trophic level than those in the North (see below).

Humpback whale diets

Humpback whales have been labeled as "fast maneuverers" as a result of their preference for fast moving, schooling fish species (Woodward *et al.* 2006), though they are often labeled as generalist in their prey selection. Feasible diet compositions modeled by IsoSource in this study, however, suggest a higher reliance on euphausiids around the Kodiak archipelago rather than any of the available fish species, such as capelin and juvenile pollock. While previous assessments of humpback whale diets have estimated that euphausiids comprise between five and 30% of the total diet (Perez & McAlister 1993, Kenney et al. 1997), model results here indicate an almost exclusive euphausiids diet in some solutions. However, model results also suggest regional differences in prey choice (Figure 5.6b).

The range of potential diet contributions of fish species generated by the models support previous studies of humpback whale foraging in the Kodiak area. Witteveen et al. (2006) estimated the removal of pollock, capelin, eulachon, sandlance, and herring by the regional population of humpback whales based on the assumption that consumption was proportion to the relative availability of these species. Stable isotope analysis supports this assumption for herring and eulachon, which represented some of the lowest proportional contributions in this study and the former (Witteveen et al. 2006). Both herring and eulachon rarely represent the most available humpback whale prey resource due to their seasonal distribution around Kodiak Island (R. Foy, National Marine Fisheries Service, pers. comm.). Thus, the decision to rerun the IsoSource models without these species may have resulted in a more accurate representation of the distribution of feasible solutions among the remaining prey species. Additionally, IsoSource model

outputs support some findings in Witteveen et al. (2008) which showed an apparent preference for capelin over juvenile walleye pollock when the availability of both species overlapped temporally and spatially within the North sampling region.

The relatively high contribution of sandlance, however, does not agree with the previous diet study (Witteveen et al. 2006). It should be noted, however, that the previous study relied on prey survey data and sandlance were likely underestimated because they are inherently difficult to sample. Thus, incorporating stable isotope analysis with traditional prey survey methodology may help to reduce or eliminate the potential exclusion of certain prey resources.

Model results suggest annual variation in the overall composition of humpback whale diets. The most consistent trend between the 2004 sampling groups and the 2005/2006 sampling groups was an increase in the range of the feasible contribution made by euphausiids. With the increase in euphausiids came a decrease in the feasible contributions of forage fish, most notably a reduction in small walleye pollock consumption. Mean δ^{15}N decreased across years, indicating humpback whales may have been feeding at a lower trophic level. Foraging at a lower trophic level would logically correspond to an increase in the contribution of euphausiids to the diet, since they represent a lower trophic level when compared to most forage fish species.

Differences in annual humpback whale diets may be the result of fluctuation in the availability of preferred prey. Evaluating regional prey abundance concurrently with stable isotope analysis of humpback whales and surveyed prey would help to determine if there is a relationship between availability and consumption. Differences in diets may be the result of changes in sample distribution across years. The extremely high proportion

of euphausiids in the 0506S diets may have resulted from the fact that over half of the 2006 samples were collected in an area where humpback whales were observed laterally echelon feeding, a behavior often associated with high concentrations of zooplankton (Jurasz & Jurasz 1979).

Finally, it is important to acknowledge that the time frame of modeled diets depends upon the turnover rate of assimilated tissues. Tissues that are more metabolically active (i.e., skin or muscle) will have a much faster turn-over rate than inert tissues (i.e., baleen or bone) (Tieszen et al. 1983, Hobson & Clark 1992b, MacAvoy et al. 2006, Podlesak & McWilliams 2006). Though never empirically tested, the skin of rorqual whales likely exhibits high metabolic rates and anecdotal evidence has suggested a turnover rate of 7-14 days for humpback whale skin (Todd 1997). Thus $\delta^{13}C$ and $\delta^{15}N$ ratios of humpback whales in this study may reflect diets of two weeks to one month prior to sampling.

Sensitivity of diet modeling

It is not likely that the choice of discrimination factors substantially impacted model results. First, factors used, ~1.3‰ for $\Delta\,\delta^{13}C$ and ~2.4‰ for $\Delta\,\delta^{15}N$, were only slightly different than generalized values often employed in dietary mixing models. Fractionation of carbon is often estimated at 0 to 1‰, while $\Delta\,\delta^{15}N$ is 3 to 5‰ (Rau et al. 1983, Fry 1988, Hobson et al. 1994, Hobson et al. 1996, Kelly 2000, Kurle & Worthy 2001, 2002). Second, of the seven models with adjusted discrimination factors, five of them were not able to reach any feasible solutions, even when tolerances values were increased to 0.2‰. The two models that were able to produce feasible results were the two models which most closely resemble the base model and in which one of the

discrimination factors remained at its original value. Overall, model results suggested nearly equal contributions of euphausiids and fish species regardless of the value of discrimination factors used.

The most pronounced change was the feasible contribution of either small walleye pollock or large capelin. Interestingly, the stable isotope ratios of these two groups did not differ significantly and selection of one over the other is often in question (Witteveen et al. 2006, Witteveen et al. 2008). Thus, discrimination factors likely have the most influence on prey groups that are isotopically similar. Determination of discrimination factors specific to cetacean skin may refine model estimates of feasible contributions in such situations.

Conclusions

Dietary information modeled in this study provided new insights into the potential composition of humpback whale diets. The iterative approach of the program IsoSource enabled exploration of feasible contributions of more than three prey sources, an approach that would not normally be permitted with the analysis of only two isotopes. The application of stable isotope analysis in this manner will be further enhanced when combined with other methods of exploring cetacean foraging ecology, such as tagging and prey surveys. Developing diet models for other feeding groups of humpback whales will permit specific comparison of regional diets. In turn, the ability to more accurately describe and compare with improved accuracy these diets in terms of resource of quality it may be critical in determining the relative survival and reproductive success of distinct foraging populations. These types of comparisons can be used when evaluating management plans and conservation efforts. Stable isotope analysis proved to be a

valuable and relatively simple means of investigating the feeding habits and prey

preferences of free-ranging cetacean species and should be implemented in foraging

studies when practical.

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Table 5.1. Mean (± SE) values of stable carbon and nitrogen isotopes and sample sizes of skin collected from free-ranging humpback whales in 2004, 2005, and 2006 and the North and South sampling regions near the Kodiak archipelago.

Year	n	$\delta^{13}C$	$\delta^{15}N$
2004	29	-17.3 ± 0.09	13.5 ± 0.10
2005	45	-18.0 ± 0.07	13.5 ± 0.15
2006	22	-18.2 ± 0.10	13.0 ± 0.14
Region			
North	42	-17.7 ± 0.08	13.7 ± 0.13
South	54	-17.9 ± 0.09	13.1 ± 0.10
Total	96	-17.9 ± 0.06	13.4 ± 0.09

Table 5.2. Mean (±SE) values of stable carbon and nitrogen isotopes and sample sizes of potential humpback whale prey categories. Prey species were collected during mid-water trawl surveys conducted near Kodiak Island between 2003 and 2005. Species with asterisk are from C. Williams (2008).

Species	n	Size Range (cm)	$\delta^{13}C$	$\delta^{15}N$
Euphausiids*	12	n/a	-19.7 ± 0.04	10.7 ± 0.11
Sm. Capelin	9	< 6	-19.8 ± 0.31	11.3 ± 0.15
Pac. Sandlance*	14	n/a	-18.4 ± 0.16	11.5 ± 0.27
Sm. Pollock + Lg. Capelin	48	6 - 12	-18.4 ± 0.07	11.6 ± 0.06
Lg. Pollock	4	12 - 22	-17.0 ± 0.17	12.9 ± 0.10
Small Eulachon	10	< 10	-16.7 ± 0.08	13.2 ± 0.09
Lg. Eulachon + Pac. Herring	44	10 - 30	-17.8 ± 0.11	13.5 ± 0.09

Table 5.3. Results of sensitivity analysis of discrimination factors in IsoSource diet modeling. Values shown are mean contributions of each of four prey species in potential diets of Kodiak Island humpback whales. For comparison the base model is listed first in the table.

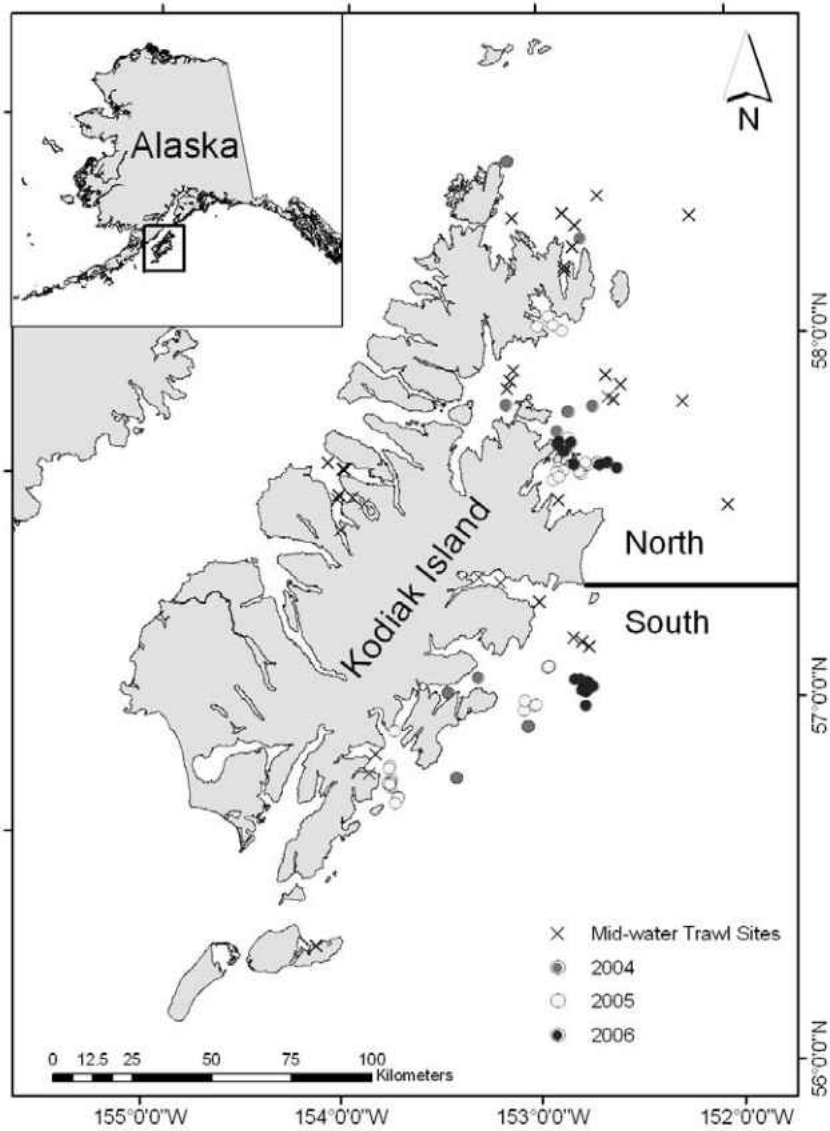| Δ			Prey Species			
$\delta^{13}C$	$\delta^{15}N$	Euphausiids	Pacific Sandlance	Large Capelin	Small Walleye Pollock
1.3	2.4	60%	14%	12%	14%
0	0		No Feasible Solutions		
0	2.4		No Feasible Solutions		
1	2.4	43%	16%	14%	27%
2	2.4		No Feasible Solutions		
1.3	0		No Feasible Solutions		
1.3	2	45%	14%	39%	2%
1.3	4		No Feasible Solutions		

Figure 5.1. Map of the Kodiak Island archipelago showing collection locations of humpback whale skin (○) for 2004, 2005, and 2006. The solid line represents the distinction between the North and South sampling regions. Also shown are tow locations of mid-water trawl conducted for prey collection (x).
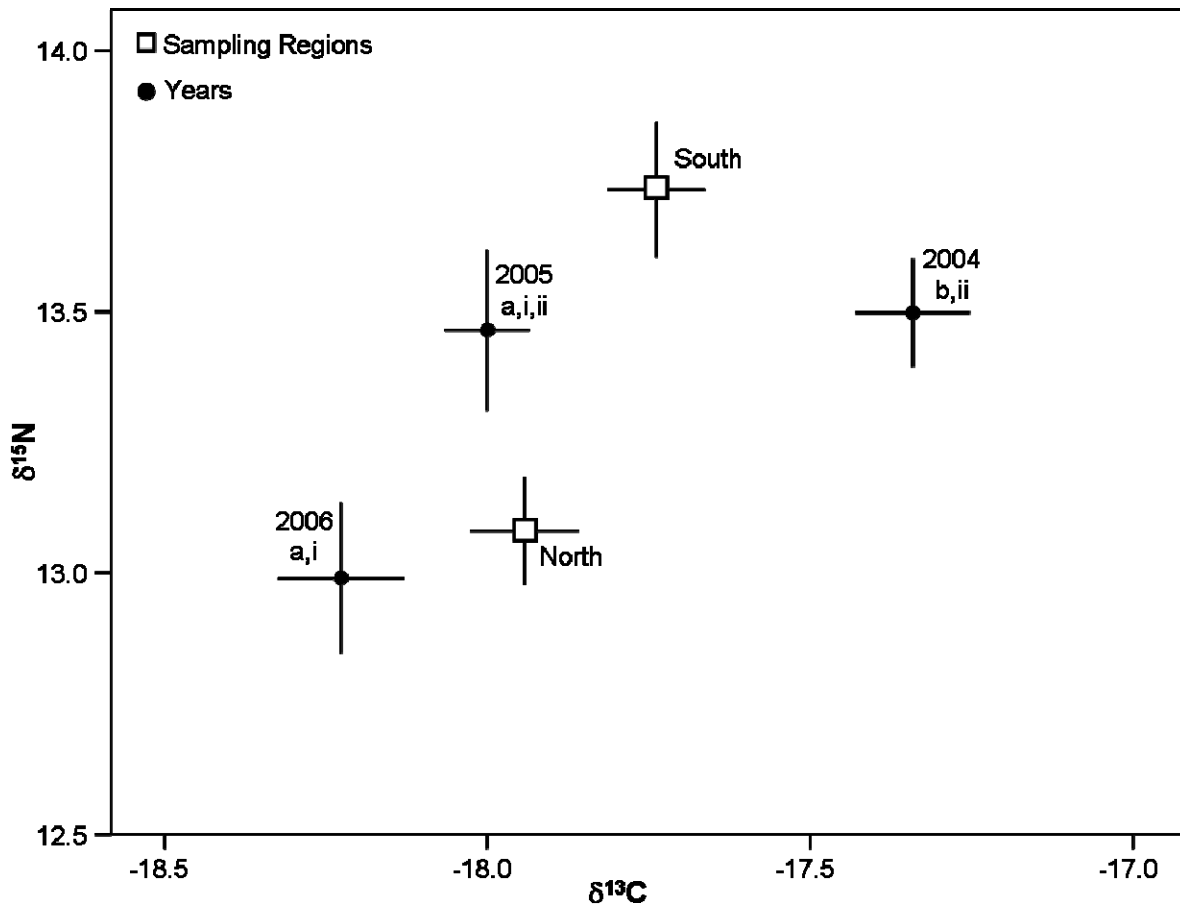
134

Figure 5.2. Mean values of $\delta^{13}C$ and $\delta^{15}N$ for Kodiak Island humpback whale skin for each of the three years (●) and sampling regions (□) in which samples were collected. Letter indicate groupings for years in which mean $\delta^{13}C$ values were not significantly different, while roman numerals indicates years in which mean $\delta^{15}N$ values were not significantly different.
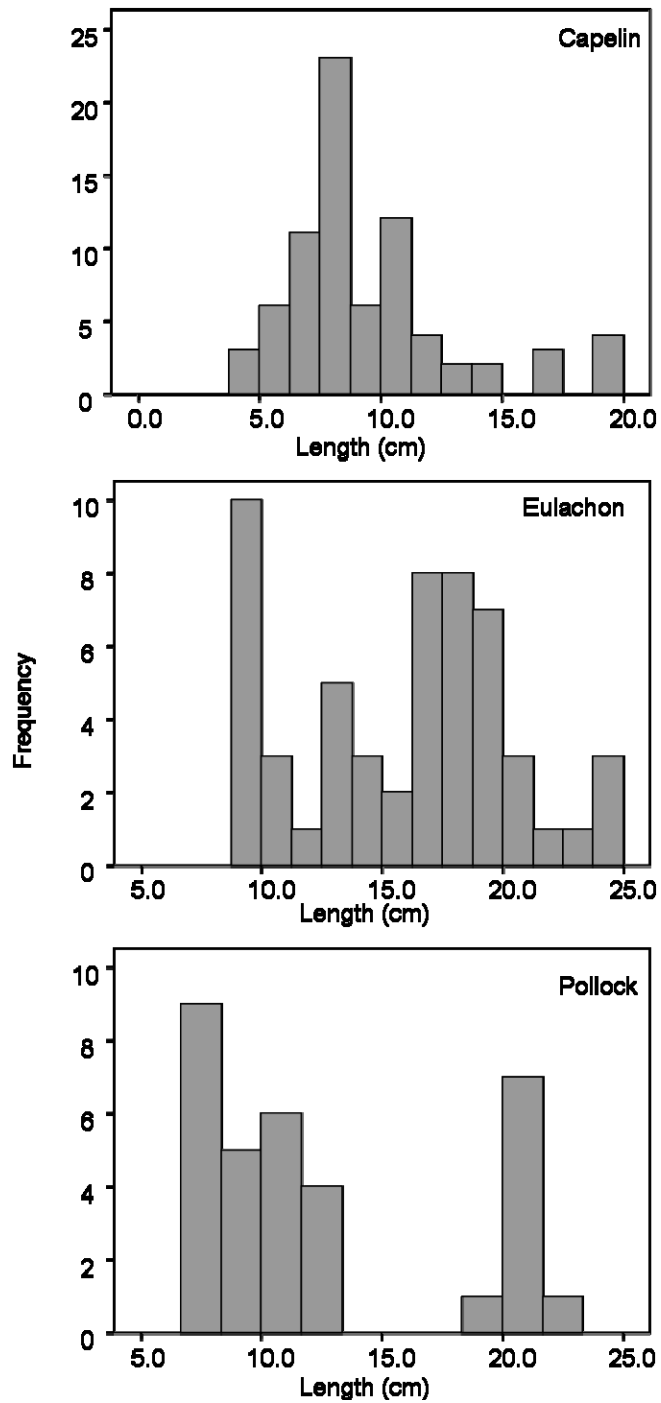
Figure 5.3. Length (cm) frequencies of capelin, eulachon, and walleye pollock collected during mid-water trawl surveys near Kodiak Island, Alaska.
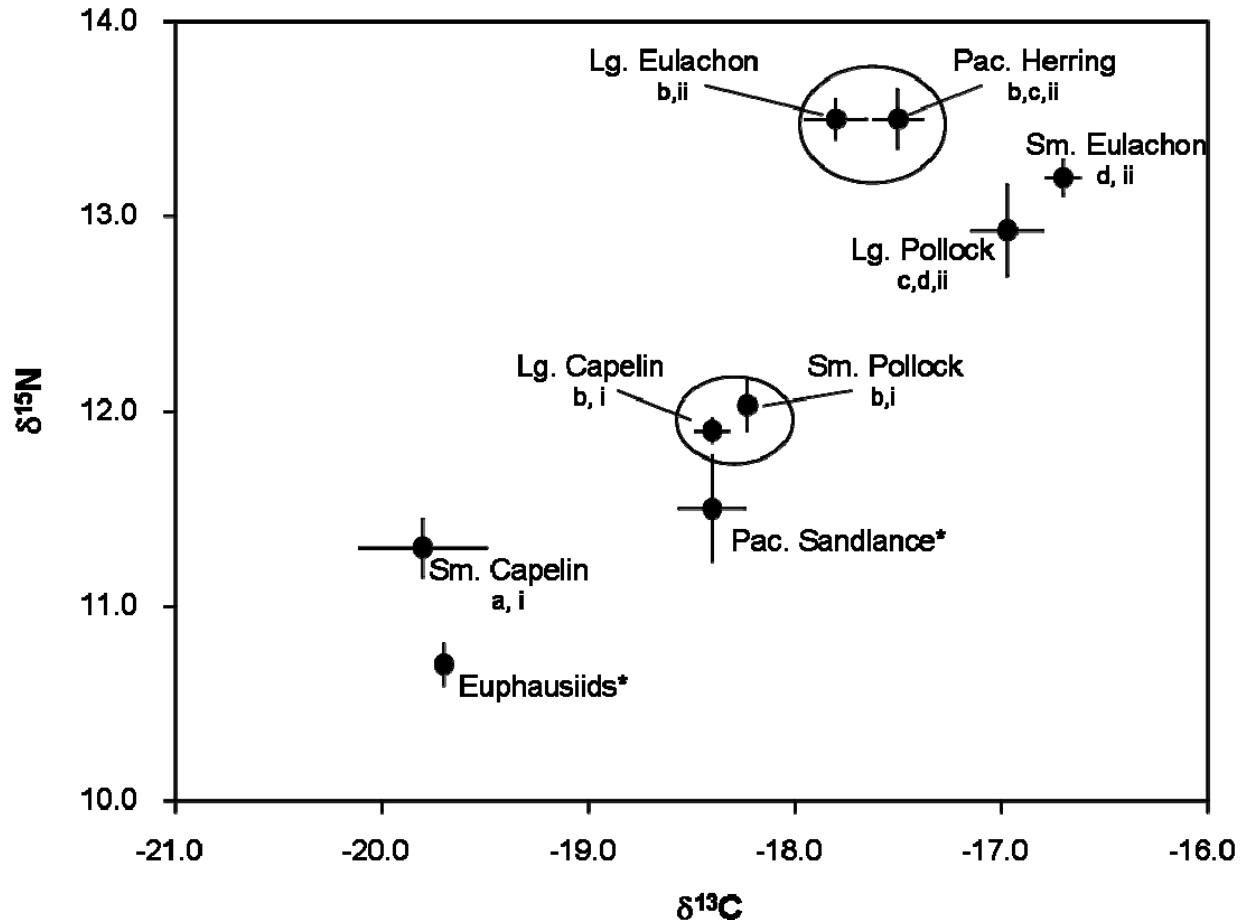
Figure 5.4. Mean values of $\delta^{13}C$ and $\delta^{15}N$ for potential Kodiak Island humpback whale prey. Samples were collected during mid-water trawl surveys. Letter indicate groupings for years in which mean $\delta^{13}C$ values were not significantly different, while roman numerals indicates years in which mean $\delta^{15}N$ values were not significantly different as shown by post-hoc tests. Ellipses surround groups that were combined in initial IsoSource diet modeling. Species with an asterisk (*) are from C. Williams (2008) and were not included variance testing.
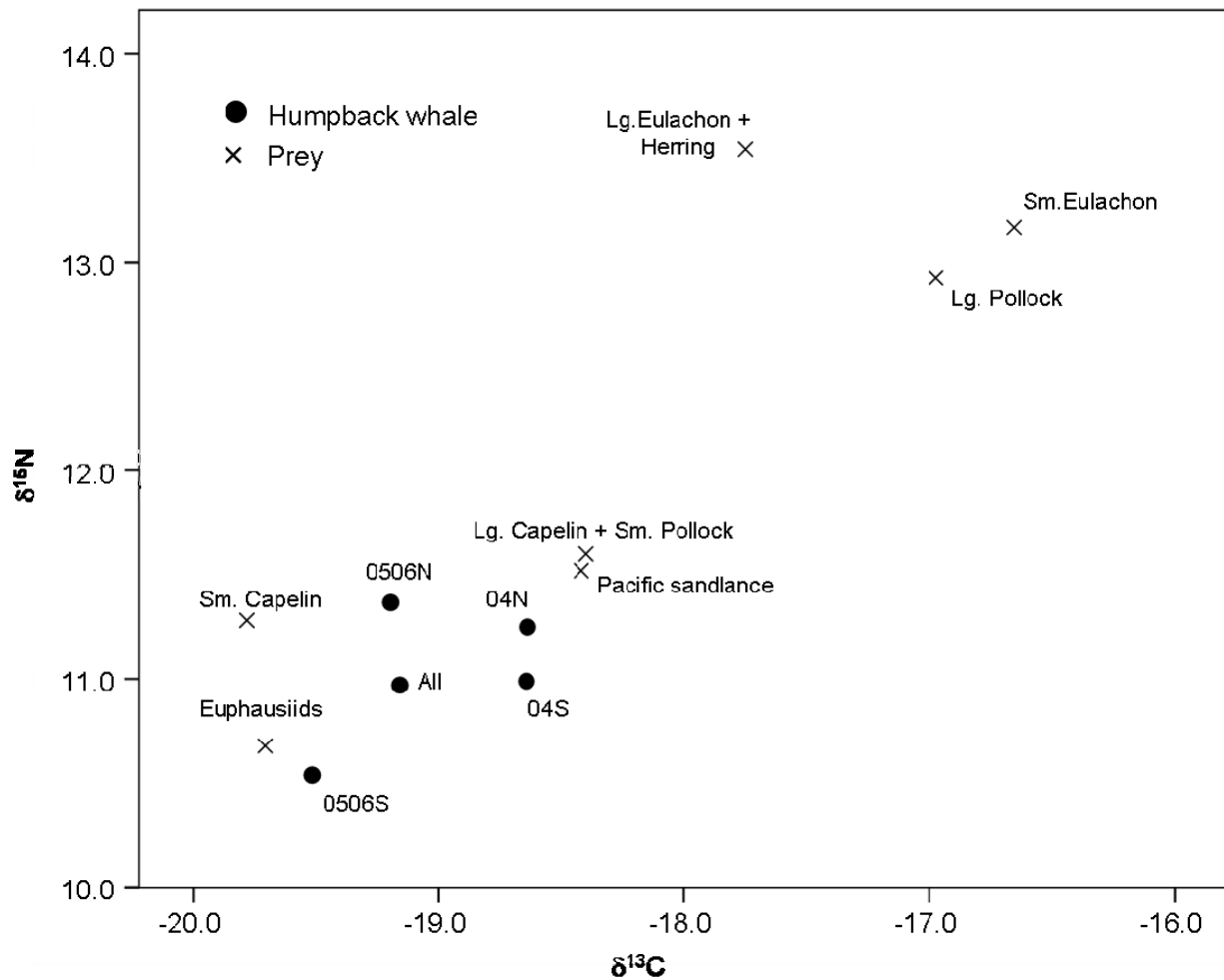
Figure 5.5. Values of $\delta^{13}C$ and $\delta^{15}N$ of humpback whale skin (●) and potential prey (x) used as input in the IsoSource mixing model. Values for sampling group (04N, 04S, 0506N, and 0506S), as well as the pooled value (All) from all humpback whale skin samples are shown.
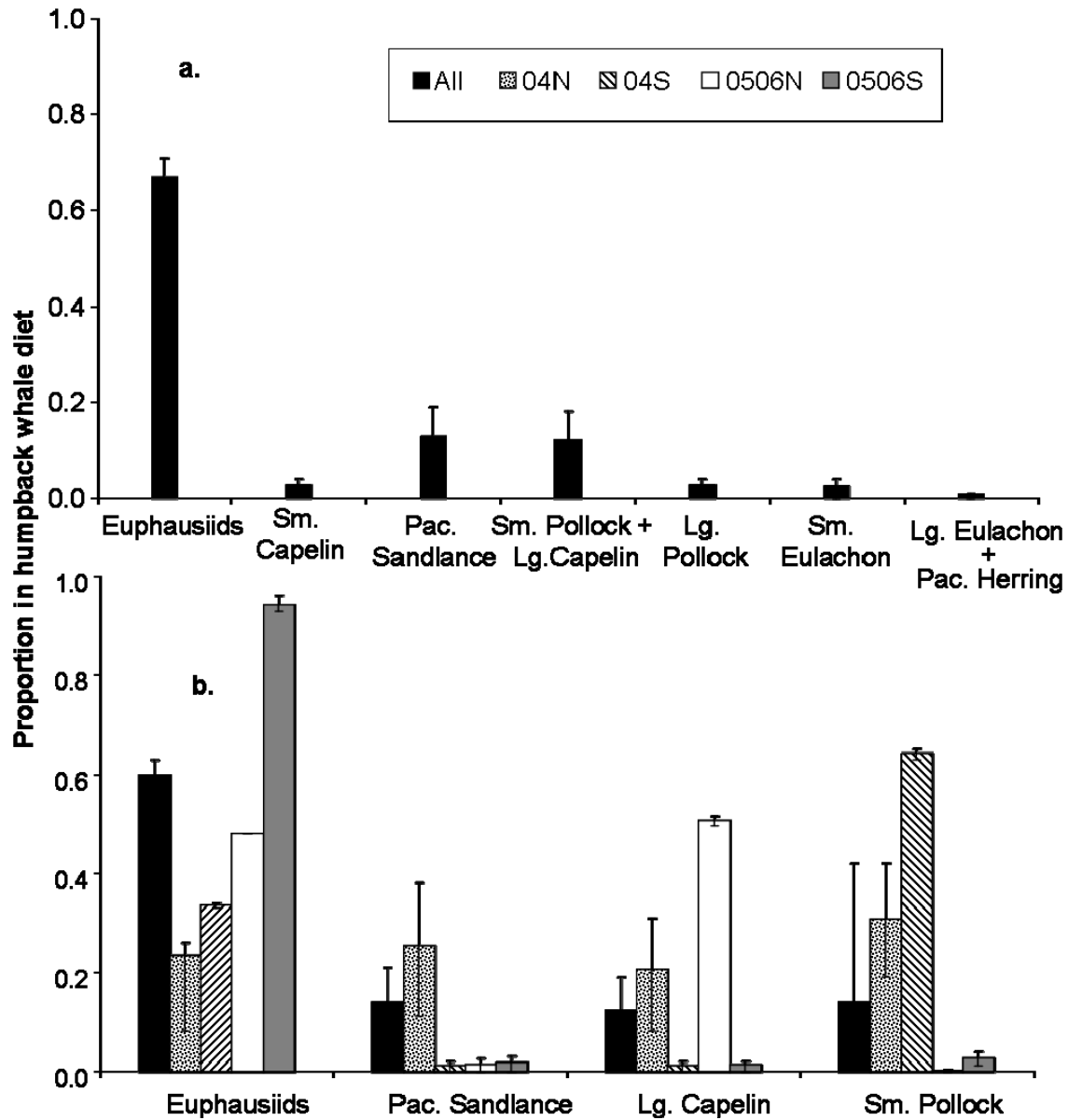
Figure 5.6. Dietary proportion for humpback whale diets in each of the four sampling groups and groups combined near the Kodiak archipelago using either all (a.) or four (b.) prey sources in the IsoSource mixing model. Values shown are means with error bars representing the 25% and 75% intervals.

CHAPTER 6: CONCLUSION

This dissertation represents the first comprehensive analysis of the population structure and foraging ecology of an entire migratory population using stable isotopes. Analysis of skin collected from North Pacific humpback whales showed ratios of stable carbon and nitrogen isotopes varied with sample location on both feeding and breeding grounds. Chapter 2 explored $\delta^{13}C$ and $\delta^{15}N$ of whales sampled on feeding grounds and supported segregation of humpback whales into distinct foraging groups (Waite et al. 1999, Calambokidis et al. 2001, Witteveen et al. 2004). Of the two isotopes analyzed, $\delta^{13}C$ reflects origin of feeding location while $\delta^{15}N$ represents relative trophic position (Hobson & Welch 1992, Rau et al. 1992, Hobson & Wassenaar 1999, Kelly 2000). Following this convention, it may be possible to identify foraging groups by $\delta^{13}C$ alone. However, results of classification analysis clearly showed that the inclusion of $\delta^{15}N$ improved model accuracy. While variability in $\delta^{15}N$ is not traditionally used to describe geographic groups, it was not surprising that inclusion of stable nitrogen isotope ratios improved group separations. The ability to identify unique groups is often enhanced by the addition of other stable isotopes (Hobson 1999).

Photo-identification of humpback whales suggests finer segregation than what was seen here (Calambokidis et al. 2008), which may simply be a factor of scale. With respect to photo-identification, segregation into feeding aggregations is defined by a lack of sightings between areas. Patterns in stable isotope ecology are the result of physical and biological processes that may not operate at scales small enough to detect such fine levels of population structure. As stated above, the ability to distinguish unique groups is enhanced with the addition of each isotope and structure detected through photo-identification may be seen with the incorporation of

another isotope, such as oxygen or hydrogen (Hobson 1999). Additional stable isotopes would likely also affect classification tree models and could improve upon the 57% accuracy rate for feeding group classification.

Results in Chapter 3 relied heavily on the assumption that stable carbon isotope ratios remained relatively unchanged on breeding grounds and accurately reflected foraging origins of humpback whales. Comparison of stable isotope ratios from animals sampled on both of their seasonal habitats provided strong support for this assumption. Differences between the stable isotope ratios of these animals were not significant. By verifying these relationships, application of the classification tree model from Chapter 2 to breeding animals was justified and described migratory movements of breeding humpback whales without knowledge of foraging location. Results of assignment based on stable isotope analysis showed strong east-west patterns and were remarkably similar to recent patterns of movement documented through photo-identification (Calambokidis et al. 2008). Further, breeding areas which showed the most diversity in feeding group assignment were the same breeding areas that showed photographic matches to several feeding groups (Calambokidis et al. 2008).

Chapter 4 presented evidence of clear differences in the trophic levels of feeding groups, suggesting some degree of prey selectivity or availability likely occurs on feeding grounds. The success of classification tree models allowed this dissertation to apply knowledge of trophic level differences of foraging animals to carry over effects on breeding grounds. While this study was not intended to describe these carry over effects, it did make progress toward describing differences in prey choice. In turn, these differences may be used to explore the impacts of prey choice and diet composition on reproductive success and survival. Detailed analysis of prey and

predator within each feeding group is needed to further expand on trophic level differences of humpback whales. Chapter 5 showed how the application of isotope mixing models can provide such details by describing potential diets for finite regions and can refine estimates of diet composition. Modeling the diet of Kodiak Island humpback whales showed that previous estimates of consumption by these animals likely overestimated the importance of juvenile walleye pollock (*Theragra chalcogramm*) and eulachon (*Thaleichthys pacificus*) and underestimated the importance of euphausiids (*Thysanoessa* spp.), capelin (*Mallotus villosus*), and Pacific sandlance (*Ammodytes hexapterus*) (Witteveen et al. 2006)

Some analyses applied in this dissertation were based on assumptions of tissue turnover rates and discrimination factors. Neither of these parameters is known for humpback whales and values estimated from other marine mammals (pinnipeds) were used as surrogates (Hobson et al. 1996, Todd 1997, Das et al. 2003). Unfortunately, both will likely continue to be a difficult estimate simply due to the nature of the study animal. Determining turnover rate or discrimination factors would require repeated sampling of an animal foraging on a known diet over a defined time scale. Clearly this is not practical for free-ranging large cetaceans, but may be possible for smaller, captive cetaceans. In the mean time, estimates from other marine mammals will have to serve as suitable substitutes.

The chapters of this dissertation make a significant contribution to the understanding of the population structure and foraging ecology of North Pacific humpback whales. Stable isotope techniques commonly used to describe components of the life history of migratory animals were successfully applied and showed the utility and benefits of such techniques for studying species'

ecology. It is likely that further benefits will be realized as these results are combined with

results from other methodologies, such as analysis of photographs and molecular markers.

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