Tracing migratory movements of breeding North Pacific humpback whales using stable isotope analysis

Briana H. Witteveen[1,3,*], Graham A. J. Worthy[1,2], James D. Roth[1]

[1]University of Central Florida, Department of Biology, 4000 Central Florida Boulevard, Orlando, Florida 32816-2368, USA
[2]Hubbs-SeaWorld Research Institute, 6295 Sea Harbor Drive, Orlando, Florida 32821, USA

[3]*Present address:* University of Alaska Fairbanks, School of Fisheries and Ocean Sciences, 118 Trident Way, Kodiak, Alaska 99615, USA

ABSTRACT: North Pacific humpback whales *Megaptera novaeangliae* are migratory animals with a complex population structure, segregating into geographically distinct aggregations on high-latitude feeding grounds. Several feeding aggregations may converge on a common breeding ground for mating and calving. Understanding how feeding and breeding habitats are linked is critical to understanding humpback whale life history and addressing management and conservation efforts. In a continued effort to explore the population structure of North Pacific humpback whales through the analysis of stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$), the present study extends on a previous study of feeding animals to describe migratory linkages to breeding grounds (Witteveen et al. 2009). Skin samples (n = 597) collected from 4 known breeding regions were analyzed for $\delta^{13}C$ and $\delta^{15}N$. Breeding regions differed in both $\delta^{13}C$ ($F_{3,585}$ = 62.3, p < 0.001) and $\delta^{15}N$ ($F_{3,585}$ = 37.2, p < 0.001). Breeding values reflected the foraging locations for 46 ind. sampled on both habitats; the relationship between the breeding and feeding stable isotope ratios was significant and positive for both $\delta^{13}C$ ($F_{1,44}$ = 10.3, r^2 = 0.19, p = 0.002) and $\delta^{15}N$ ($F_{1,44}$ = 40.9, r^2 = 0.48, p < 0.001). Furthermore, individual breeding and feeding values did not differ for $\delta^{15}N$ (t_{45} = 1.41, p = 0.17) or $\delta^{13}C$ (t_{45} = –1.15, p = 0.26) in pairwise comparisons. We used $\delta^{13}C$ and $\delta^{15}N$ in a classification tree analysis to describe probable migratory linkages to 6 previously described feeding groups. Stable isotope ratios predicted regional patterns of movement, and assignments of breeding individuals to feeding grounds differed by 12% on average from photographic matching. Our results indicate this technique can be used to help understand the population structure and ecology of North Pacific humpback whale populations, especially when used in combination with other research techniques.

KEY WORDS: Classification tree analysis · *Megaptera novaeangliae* · Migration · Stable isotopes

INTRODUCTION

Migration has evolved independently among a number of animal taxa, including birds, ungulates, and marine mammals, often in response to seasonally advantageous habitat characteristics such as concentration of prey or refuge from predators during calving (Aidley 1981, Corkeron & Connor 1999, Alerstam et al. 2003). The North Pacific humpback whale *Megaptera novaeangliae* is a 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 engage in little or no feeding (Dawbin 1966, Lockyer 1981, Baraff et al. 1991, Laerm et al. 1997).

North Pacific humpback whales segregate into geographically distinct aggregations while on their summer feeding grounds. While very little exchange

occurs between these aggregations, several aggregations may converge on a common winter breeding ground (Calambokidis et al. 1996, Waite et al. 1999, Urbán 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 British Columbia and Alaska (Baker et al. 1990, Perry et al. 1990, Calambokidis et al. 1997); humpback whales using Japanese waters in winter migrate to Russia and the Bering Sea

Table 1. *Megaptera novaeangliae*. The 6 feeding groups as defined by previous stable isotope analysis and mean values of δ^{13}C and δ^{15}N ($\pm$SE). Adapted from Witteveen et al. (2009)

Feeding group	Regions included	δ^{13}C	δ^{15}N
WEST	W. Aleutian Islands, Russia	−17.8 ± 0.10	12.2 ± 0.19
CENT	E. Aleutian Islands, Bering Sea, W. Gulf of Alaska	−18.4 ± 0.04	12.5 ± 0.07
NGOA	N. Gulf of Alaska	−17.6 ± 0.05	13.5 ± 0.07
SEAK	Southeastern Alaska	−17.1 ± 0.05	12.7 ± 0.06
NBC	N. British Colombia	−17.6 ± 0.06	12.9 ± 0.08
COW	California, Oregon, Washington	−16.4 ± 0.04	14.6 ± 0.07
Overall		−17.5 ± 0.03	13.1 ± 0.04

(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). The main or dominant migratory destination for humpback whales from a breeding ground offshore of Mexico (Revillagigedos Islands) remains unknown, though some animals have been sighted from feeding grounds from California through the Aleutian Islands (Urbán et al. 2000, Calambokidis et al. 2001, Witteveen et al. 2004, Lagerquist et al. 2008).

The life history of North Pacific humpback whales is, therefore, quite complex, and many questions remain about their population structure. Unanswered questions about migratory destinations, routes, and habitat usage inhibit management and conservation efforts. Research focused on linking seasonal habitats is needed in order to address this issue. Traditional techniques used to identify migratory connections, including photo-identification (i.e. Urbán 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 ratios of an animal's tissues reflect those 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 at their breeding grounds, the isotopic signatures of foraging grounds should be retained throughout the breeding season.

Previous analysis of carbon and nitrogen stable isotope signatures of foraging humpback whales defined 6 isotopically distinct feeding groups in the North Pacific using classification tree analysis (Table 1, Figs. 1 & 2; Witteveen et al. 2009). In the present study, results from the classification tree analysis were applied to investigate relationships of individuals sampled on breeding grounds and to assign breeding individuals to 1 of the 6 previously designated feeding groups. Results can be used to provide insight into the complex population structure and ecology of North Pacific humpback whale populations without having to sample or photograph the same individual at both habitats.

MATERIALS AND METHODS

Sample collection. Samples for isotopic analysis were collected from free-ranging humpback whales *Megaptera novaeangliae* throughout all known breeding regions in the North Pacific basin, as part of the Structure of Populations, Level of Abundance, and Status of Humpback whales (SPLASH) project. Effort was divided into 4 sampling regions. Sampling regions were defined based on the distribution of humpback whale breeding grounds, areas of pre-existing research effort, and availability of researchers. Sampling regions were defined as Asia, Hawaii, Mexico, and Central America (Fig. 1). Since sampling regions were fairly broad, breeding areas within some sampling regions were defined by SPLASH protocol. Sample collection occurred on 5 Hawaiian Islands, but comprised just a single breeding area. For Mexico, breeding areas were the Baja Peninsula (Baja Pen), mainland Mexico (Main Mex), and the offshore Revil-
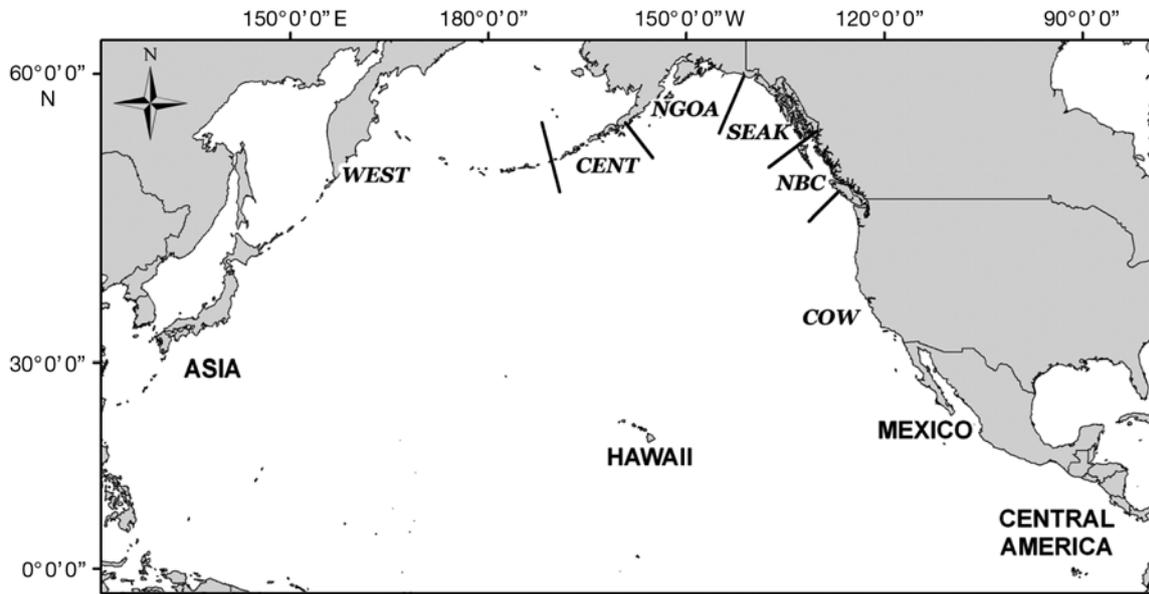
Fig. 1. Map of the North Pacific Ocean showing the 4 regions (Asia, Hawaii, Mexico, and Central America) of SPLASH (Structure of Populations, Level of Abundance, and Status of Humpback whales) sampling on breeding grounds of humpback whales *Megaptera novaeangliae*. Also shown are the 6 feeding groups (in italics, see Table 1) defined previously (Witteveen et al. 2009), with solid lines representing approximate group borders
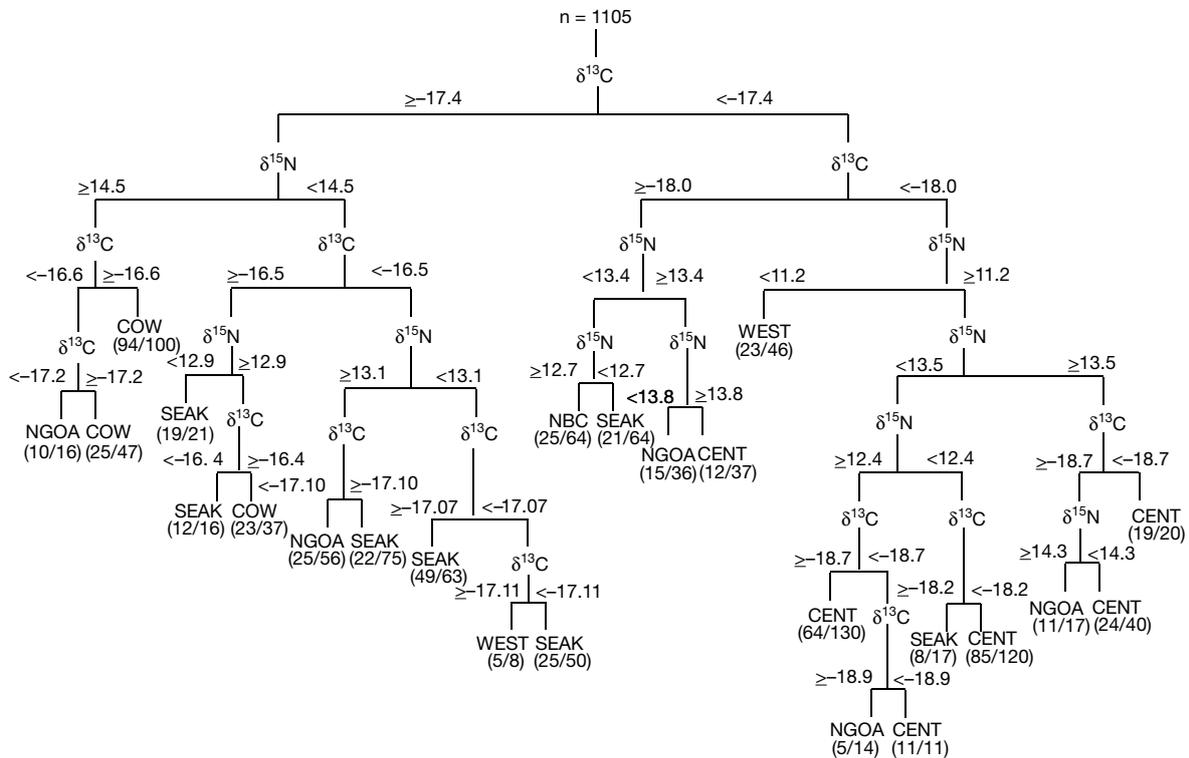

Fig. 2. *Megaptera novaeangliae*. Classification tree model used to assign individual samples in the breeding areas to 1 of 6 feeding groups (from Witteveen et al. 2009). Rationale for each split is indicated by δ values. Labels at the terminal nodes indicate the dominant feeding group at that number (numbers reflect the proportion of samples assigned to that node that belong to that feeding group)

lagigedos Islands (Rev Is.) and for Asia, the areas were Ogasawara and Okinawa, Japan, and the Philippines. Although Costa Rica, Nicaragua, and Guatemala were separate sampling locations within Central America, we considered them a single breeding area because of the small sample size (Fig. 3).

Sampling effort occurred between 9 January and 1 May for the 2004 breeding season, between 19 December 2004 and 13 May for the 2005 breeding season, and between 10 January and 1 May for the 2006 breeding season (Calambokidis et al. 2008).

Sample collection and preservation followed methods detailed in Witteveen et al. (2009). Briefly, skin samples were collected using a biopsy darting system or from sloughed skin. Whenever possible, photographs of the tail flukes of sampled individuals were also collected at each sampling event. Additional data recorded included the date, location (latitude and longitude), and general whale behavior.

Sample preparation and stable isotope analysis. Skin samples were prepared for stable isotope analysis through a multi-step process that included oven dry-

ing, extraction of lipids, and homogenization (Witteveen et al. 2009). 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 by 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 ±0.01 SE for both δ^{13}C and δ^{15}N based on 202 test standards (bovine tissue).

Statistical analysis. All statistics were conducted using SPSS 15.0 or JMP 7.0 for Windows with a critical value of $\alpha = 0.05$. We tested our data for normality and homogeneity of variance and compared differences in stable isotope ratios among sampling regions and years using factorial analysis of variance (ANOVA),
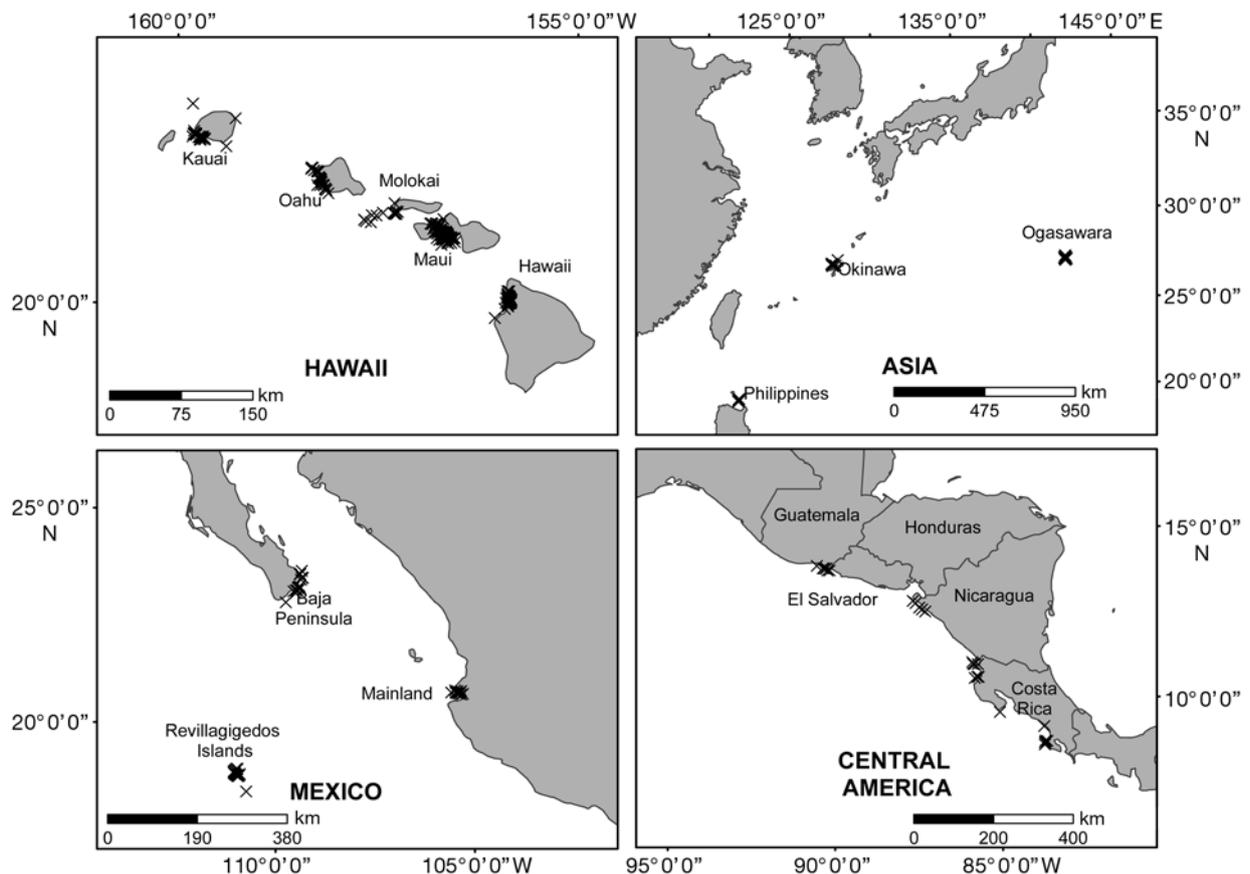

Fig. 3. *Megaptera novaeangliae.* Maps of breeding areas within each of the 4 SPLASH sampling regions. Locations of sample collections (×) are also shown

run separately for $\delta^{13}C$ and $\delta^{15}N$. Homogeneous subsets were determined using Tukey's post hoc tests. The sex of 202 ind. was known (60 females and 142 males). Sexes did not differ for either carbon ($F_{1,200} = 0.19$, p = 0.66) or nitrogen ($F_{1,200} = 0.01$, p = 0.91) ratios when controlling for sampling region and year, so sex was excluded as a factor in subsequent analyses. Breeding areas within Asia and Mexico, 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 $\delta^{15}N$ were analyzed in order to describe relationships between areas without considering sampling region.

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 as the predictor variable. In addi-

tion, ratios were compared using paired sample *t*-tests to determine if measured stable isotope ratios differed significantly between the 2 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 (Witteveen et al. 2009) was applied to breeding area samples (Fig. 2). The classification model incorporated $\delta^{13}C$ and $\delta^{15}N$ as variables to predict foraging location for individuals sampled on feeding grounds. Feeding groups were defined as California, Oregon, and Washington (COW), northern British Columbia (NBC), southeastern Alaska (SEAK), northern Gulf of Alaska (NGOA), western Gulf of Alaska, eastern Aleutian Islands, and Bering Sea (CENT), and western Aleutian Islands and Russia (WEST) (Fig. 1, Table 1; Witteveen et al. 2009). The model was applied to breeding samples to determine the success of the model at assigning individuals to 1 of the 6 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. Assignments of breeding individuals 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 verified migratory links (Calambokidis et al. 2008).

Table 2. *Megaptera novaeangliae*. Sample sizes for stable isotope analysis by year for each sampling region and for each breeding area within a region

Region	—— Year ——			Total
Breeding area	2004	2005	2006	
Asia				
Ogasawara	38	42	23	103
Okinawa	0	2	20	22
Philippines	6	6	0	12
Total	44	50	43	137
Hawaii				
Hawaiian Is.	124	137	49	310
Mexico				
Rev Is.	17	25	13	55
Baja Pen	19	0	14	33
Main Mex	14	0	15	29
Total	50	25	42	117
Central America				
Cent Am	9	10	14	33
Total	227	222	148	597

RESULTS

We measured stable isotope ratios in 597 samples of humpback whale *Megaptera novaeangliae* collected by the SPLASH project over 3 yr. Differences in sample sizes among regions generally reflect variability in sampling effort (Table 2). For all sampling regions combined, the mean value of $\delta^{13}C$ was -17.8 ± 0.04 and the mean value of $\delta^{15}N$ was 12.9 ± 0.06 (Table 3). Regional mean values of $\delta^{13}C$ ranged from a high of

Table 3. *Megaptera novaeangliae*. 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

| Region	$\delta^{13}C$			Overall mean	$\delta^{15}N$			Overall mean
	2004	2005	2006		2004	2005	2006	
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
Hawaii	-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
Mexico	-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
Central America	-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	$-17.6 \pm 0.07a$	$-18.1 \pm 0.06b$	$-17.8 \pm 0.08c$	-17.8 ± 0.04	$13.0 \pm 0.10a$	$12.8 \pm 0.09b$	$13.1 \pm 0.14a$	12.9 ± 0.06
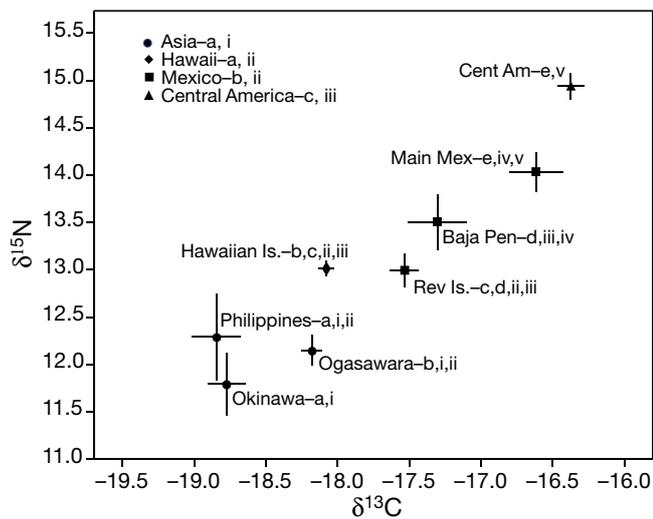
Fig. 4. *Megaptera novaeangliae*. Mean values (±SE) of $\delta^{15}N$ and $\delta^{13}C$ for each breeding area (see Fig. 3), with symbols representing sampling region membership. Letters indicate areas that are similar with respect to $\delta^{13}C$, while Roman numerals indicate similarities with respect to $\delta^{15}N$

–16.3 ± 0.14 for Central America to a low of –18.3 ± 0.06 for Asia. This pattern held for nitrogen values; the highest mean was Central America (14.9 ± 0.13) and lowest was Asia (12.1 ± 0.13).

$\delta^{13}C$ 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 by the interaction between the 2 ($F_{6, 585} = 1.9$, p = 0.07). With respect to $\delta^{15}N$, 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 3 homogenous subsets with respect to both carbon and nitrogen stable isotope ratios. For $\delta^{13}C$, Asia and Hawaii were not significantly different from one another, while Mexico and Central America were distinct from all sampling regions. Central America and Asia were distinct with respect to $\delta^{15}N$, while Hawaii and Mexico could not be distinguished (Fig. 4).

Asian breeding areas differed in $\delta^{13}C$ ($F_{2, 134} = 10.3$ p < 0.001), with the Philippines and Okinawa creating 1 group separate from Ogasawara, but they did not differ in $\delta^{15}N$ ($F_{2, 134} = 0.5$, p = 0.59). In Mexico, breeding areas differed for both $\delta^{13}C$ ($F_{2, 114}$ 8.1, p < 0.001) and $\delta^{15}N$ ($F_{2, 114} = 5.5$, p = 0.005). Post hoc tests for $\delta^{13}C$ separated the Rev Is. and Baja Pen from Main Mex, but not from one another. Slightly different groupings were seen in $\delta^{15}N$, with Baja Pen grouped with both Rev Is. and Main Mex and the latter 2 separated from one another.

Carbon stable isotope ratios differed significantly among breeding areas when sampling region was not considered ($F_{7, 589} = 34.9$, p < 0.001), with 5 homogeneous subgroups that generally followed regional patterns (Fig. 4). $\delta^{15}N$ differed among breeding areas as well ($F_{7, 589} = 18.9$, p < 0.001), though differentiation among breeding areas was less definitive than that of $\delta^{13}C$ (Fig. 4).

Our sample included 46 ind. that were sampled on both feeding and breeding grounds, as identified by fluke photographs. Stable isotope ratios from the breeding and feeding grounds were positively related for both $\delta^{13}C$ ($F_{1, 44} = 10.3$, $r^2 = 0.19$, p = 0.002) and $\delta^{15}N$ ($F_{1, 44} = 40.9$, $r^2 = 0.48$, p < 0.001; Fig. 5). By including time (d) and distance (km) between sampling events,

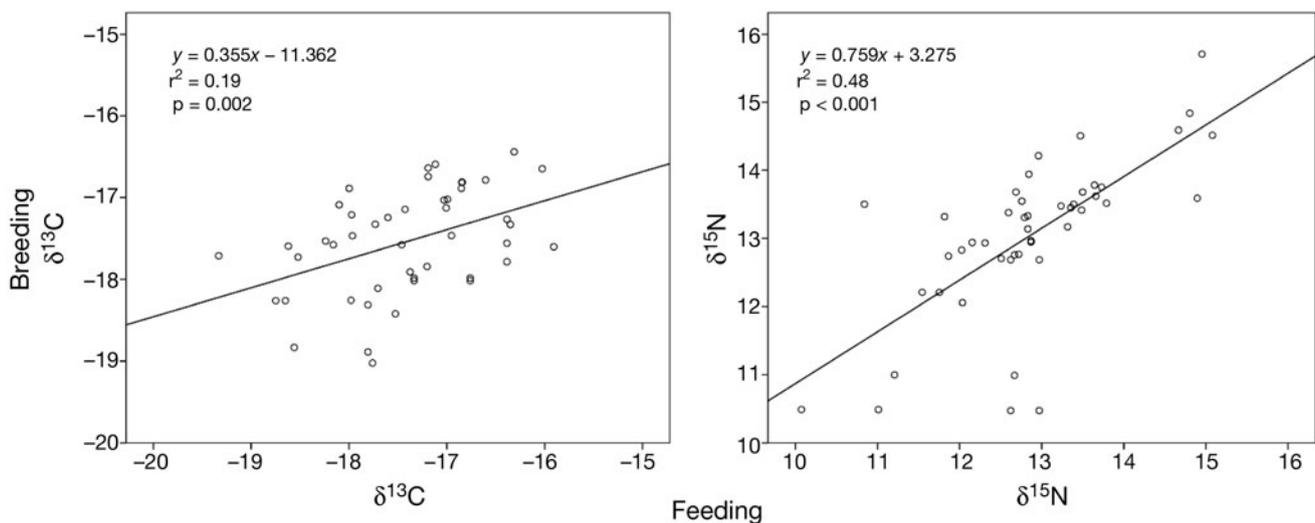Fig. 5. *Megaptera novaeangliae*. Relationships between feeding and breeding values of $\delta^{13}C$ (‰) and $\delta^{15}N$ (‰) for 46 ind. humpback whales sampled in both breeding and feeding habitats. Each point represents a single animal

only minimally improved regression results. Breeding and feeding ground samples did not differ in either $\delta^{13}C$ ($t_{45} = -1.15$, p = 0.26) or $\delta^{15}N$ ($t_{45} = 1.41$, p = 0.17) when paired samples for individuals were compared.

The classification tree model originating from $\delta^{13}C$ and $\delta^{15}N$ of humpback whales sampled on feeding grounds resulted in successful assignment of 57 % of cases (Witteveen et al. 2009; Fig. 2). This classification tree was then used to assign breeding individuals to feeding groups, and results were summarized by sampling region and breeding area. The highest proportion for each sampling region was as follows: Asia to WEST (38 %), Hawaii to CENT (36 %), Mexico to COW (31 %), and Central America 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 the breeding area to feeding group comparisons (Fig. 6). Strong connections were seen between the Philippines and CENT (57 %), Okinawa and WEST (48 %), and Baja Pen, Main Mex, and Cent Am and COW (34, 60, and 80 %, respectively) (Fig. 6).

SPLASH analysis of fluke photographs of humpback whales resulted in 873 matches between feeding grounds and breeding grounds (Calambokidis et al. 2008). The distribution of these photographic matches differed by 12 %, on average, from assignments of breeding individuals to feeding grounds based on classification tree analysis of stable isotope ratios. Some breeding areas exhibited strong average agreement between classification tree and photographic results, e.g. Baja Pen, Main Mex, and Cent Am differed by only 8, 7, and 7 %, respectively. The highest discrepancies were found for the Philippines (24 %) and Rev Is. (14 %). In some cases, classification tree assignment was nearly identical to results from photographic matching. For example, for individuals sampled in Baja Pen, 7 % were assigned to the NBC feeding groups as per classification tree analysis, which was the same percentage of Baja Pen photographs that were matched to that feeding group (Fig. 6).
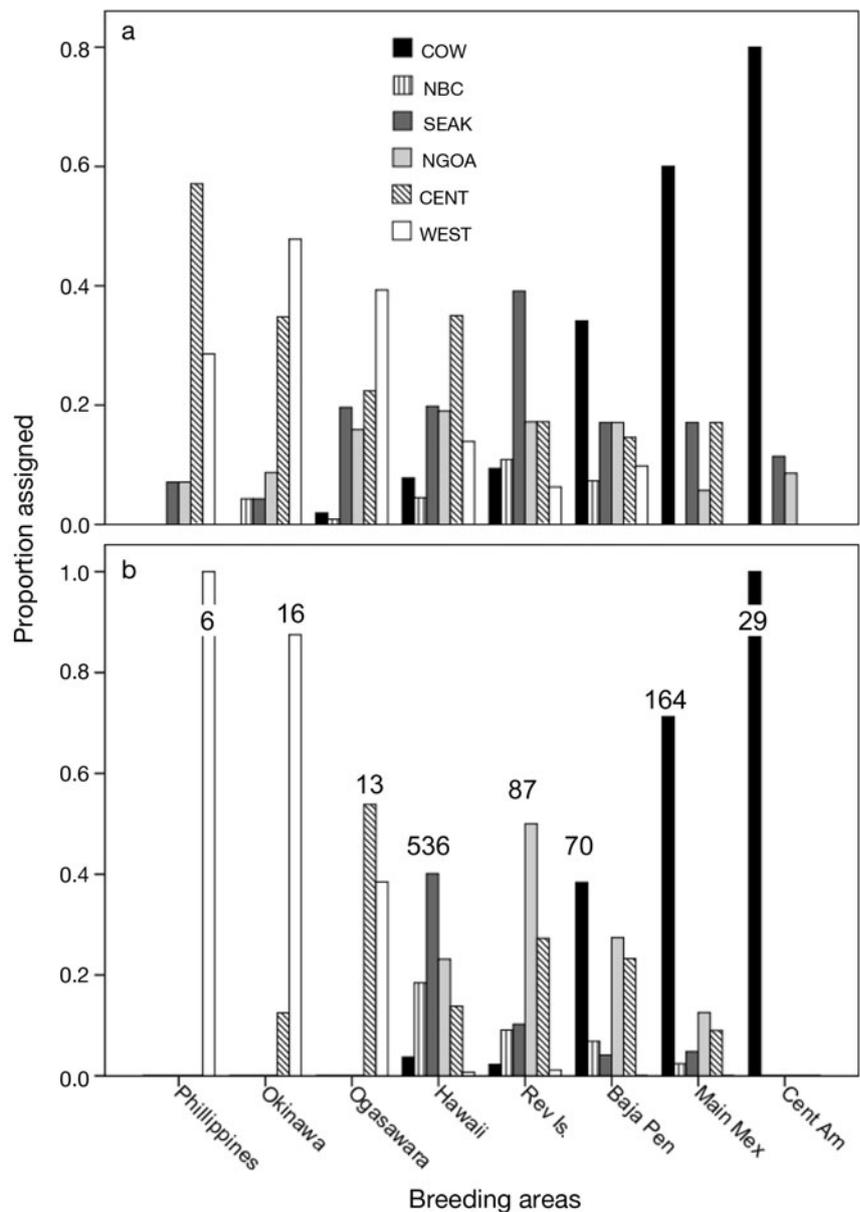

Fig. 6. *Megaptera novaeangliae*. Distribution of breeding area animals assigned to feeding groups based on (a) classification tree analysis of stable isotope ratios and (b) SPLASH photographic matching. Numbers indicate the total number of photographic matches made for each breeding area (Calambokidis et al. 2008)

DISCUSSION

Analysis of $\delta^{13}C$ and $\delta^{15}N$ in humpback whale *Megaptera novaeangliae* skin is 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 (Witteveen et al. 2009). Broadly, our results 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 1981, Baraff et al. 1991, Laerm et al. 1997), and, as such, $\delta^{13}C$ should preserve the location of most recent foraging, while $\delta^{15}N$ shows the trophic level of foraging (Gannes et al. 1997, Hobson 1999, 2006, Kelly 2000, Post 2002, Rocque et al. 2006). The present results suggest little change in stable isotope ratios between habitats and support the assumption that $\delta^{13}C$ and $\delta^{15}N$ of breeding animals remain relatively static until foraging resumes.

If $\delta^{13}C$ reflects origins of feeding, significant differences between years may be due to interannual changes in the base of the food chain on the higher latitude feeding ground, which is established at the start of each season (Saupe et al. 1989). Differences in $\delta^{13}C$ among sampling regions suggest that humpback whales breeding in Hawaii and Asia waters forage within the same geographic location, and Mexico and Central America individuals forage on distinct grounds. 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 $\delta^{13}C$ does serve as an indicator of feeding origins, a complex pattern of movement between breeding and feeding grounds can be inferred from our results, which suggest that whales from the Philippines and Okinawa migrate to isotopically similar feeding grounds, as do Main Mex and Cent Am whales. The overlap among the remaining breeding areas implies that individuals 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 signatures for these 2 breeding areas. Such movements have been documented previously by photo-identification analysis (Urbán et al. 2000, Calambokidis et al. 2001, Witteveen et al. 2004, Lagerquist et al. 2008). Further, carbon signatures on breeding grounds that have been shown to host animals from a number of feeding grounds may be confounded by the influx of carbon from a multiple feeding sources (the assignment of breeding animals to feeding groups is discussed below).

Unlike $\delta^{13}C$, $\delta^{15}N$ 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 do not forage on breeding grounds, differences in stable nitrogen isotope ratios may reflect differences in trophic position between feeding groups. Our results 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, true differences in trophic level cannot be determined without establishing the $\delta^{15}N$ value at the base of regional food webs (Schell et al. 1998, Post 2002).

In some animals, differences in $\delta^{15}N$ between feeding and breeding groups may reflect fasting (Hobson et al. 1993, Cherel et al. 2005). $\delta^{15}N$ becomes enriched by 3 to 4‰ with each trophic level in a food web and often is used to indicate relative trophic position (Hobson et al. 1994, Post 2002). While on breeding grounds, a humpback whale survives on blubber reserves accrued during foraging and essentially feeds on itself, a higher trophic level than the fish or zooplankton of a typical diet. Therefore, if stable nitrogen isotope ratios of breeding animals do 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 2 nitrogen stable isotope ratios, the values themselves were not significantly different. Other studies of fasting mammals and birds have similarly found a lack of enrichment in $\delta^{15}N$ (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 do not become 'nutritionally 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).

Studies using the stable isotope analysis often depend on the turnover rate of the tissues involved. Tissues that are more metabolically active (i.e. skin or muscle) will have a much faster turnover rate than inert tissues (i.e. baleen or bone) (Tieszen et al. 1983, Hobson & Clark 1992, MacAvoy et al. 2006, Podlesak & McWilliams 2006). The turnover rate of humpback whale skin has never been measured, but a rate of approximately 7 to 14 d has been estimated (Todd 1997). However, the individuals in the present study are assumed to have ceased foraging, and the time between sampling on feeding and breeding grounds was always >14 d. Thus, no new isotope information should be incorporated into the tissues in this analysis, and stable isotope ratios should reflect the last 14 to 28 d of foraging history.

It should be noted that, as the result of changes in annual sampling effort, sample size was small for some regions (i.e. Philippines and Cent Am) and highly variable for others (i.e. Main Mex and Baja Pen). Pooling data across years may have prevented changes in the stable isotope ratios on the feeding grounds associated with these regions to be detected.

Turning our attention to the assignment of breeding whales 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 western-most feeding groups (CENT and WEST). 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 individuals from nearly all breeding areas, however. There are a number of possible explanations for these results. First, these 2 feeding groups may truly not be dominated by any single breeding area and may serve as the feeding grounds for individuals 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 (Witteveen et al. 2009). These 2 feeding groups exhibited the fewest number of correct classifications on feeding grounds. A weakness in the model's ability to discriminate these groups would carry over into the assignment of breeding animals.

Whales were correctly assigned in 56% of cases, which was a 3.2 times higher rate 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 a strong consensus between the classification tree results and the 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 relay an accurate picture of connectivity, 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 drives the assignments. For example, Okinawa and the Philippines showed nearly identical $\delta^{13}C$ means and yet Okinawa was more frequently assigned to WEST, and the Philippines to CENT. Thus, $\delta^{15}N$ may be more influential for these areas.

Several breeding areas showed more diversity in assignments than the others. For example, the 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 stable isotope ratio values for these areas tended to be intermediate compared to other areas and were often grouped together in post hoc tests. The similarity and relative position of these means may hamper the classification tree's ability to assign these breeding areas to

a single feeding group. Alternatively, the diverse classification of these breeding areas may accurately reflect substantial mixing of feeding groups at these locations. 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 et al. 2000, Mizroch et al. 2004).

Overall, our results show promise in 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 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 a resighting requirement. When combined with other research methods, stable isotope analysis can further our understanding of the life history of North Pacific humpback whales.

LITERATURE CITED

Aidley DJ (1981) Animal migration. Cambridge University Press, Cambridge

Alerstam T, Hedenstrom A, Akesson S (2003) Long-distance migration: evolution and determinants. Oikos 103: 247–260

Aurioles D, Koch PL, Le Boeuf BJ (2006) Differences in foraging location of Mexican and California elephant seals: evidence from stable isotopes in pups. Mar Mamm Sci 22: 326–338

Baker CS, Herman LM, Perry A, Lawton WS and others (1986) Migratory movement and population structure of humpback whales (*Megaptera novaeangliae*) in the central and eastern North Pacific. Mar Ecol Prog Ser 31:105–119

Baker CS, Palumbi SR, Lambertsen RH, Weinrich MT, Calambokidis J, O'Brien SJ (1990) Influence of seasonal migration on geographic distribution on mitochondrial DNA haplotypes in humpback whales. Nature 344: 238–240

Baraff LS, Clapham PJ, Mattila DK, Bowman RS (1991) Feeding behaviour of a humpback whale in low latitude waters. Mar Mamm Sci 7:197–202

Ben-David M, McColl CJ, Boonstra R, Karels TJ (1999) N-15 signatures do not reflect body condition in Arctic ground squirrels. Can J Zool 77:1373–1378

Berzin AA, Rovnin AA (1966) The distribution and migrations of whales in the northeastern part of the Pacific, Chukchi and Bering Seas. Izv Tikhookean Nauchno-Issled Institut Rybn Khoz Okeanogr 58:179–207

Best PB, Schell DM (1996) Stable isotopes in southern right whale (*Eubalaena australis*) baleen as indicators of seasonal movements, feeding and growth. Mar Biol 124: 483–494

Calambokidis J, Steiger GH, Cubbage JC, Balcomb KC, Bloedel P (1989) Biology of humpback whales in the Gulf of Farallones, California. Gulf of Farallones National Marine Sanctuary, San Francisco, CA

Calambokidis J, Steiger GH, Evenson JR (1993) Photographic identification and abundance estimates of humpback whales off California in 1991–92. Report No. Final Report N0ABNF110137, Report to Southwest Fisheries Science Center, National Marine Fisheries Service, La Jolla, CA

Calambokidis J, Steiger GH, Evenson JR, Flynn KR and others (1996) Interchange and isolation of humpback whales off California and other North Pacific feeding grounds. Mar Mamm Sci 12:215–226

Calambokidis J, Steiger GH, Straley J, Quinn TJ II and others (1997) Abundance and population structure of humpback whales in the North Pacific Basin. Report to Southwest Fisheries Science Center, National Marine Fisheries Service, La Jolla, CA

Calambokidis J, Steiger GH, Straley JM, Herman LM and others (2001) Movements and population structure of humpback whales in the North Pacific. Mar Mamm Sci 17: 769–794

Calambokidis J, Falcone EA, Quinn TJ II, Burdin AM and others (2008) SPLASH: structure of populations, levels of abundance and status of humpback whales in the North Pacific. Final report for Contract AB133F-03-RP-00078, U.S. Dept of Commerce Western Administrative Center, Seattle, WA

Cerling TE, Wittemyer G, Rasmussen HB, Vollrath F, Cerling CE, Robinson TJ, Douglas-Hamilton I (2006) Stable isotopes in elephant hair document migration patterns and diet changes. Proc Natl Acad Sci USA 103:371–373

Cherel Y, Hobson KA, Bailleul FR, Groscolas R (2005) Nutrition, physiology, and stable isotopes: new information from fasting and molting penguins. Ecology 86:2881–2888

Cherel Y, Phillips RA, Hobson KA, McGill R (2006) Stable isotope evidence of diverse species-specific and individual wintering strategies in seabirds. Biol Lett 2:301–303

Corkeron PJ, Connor RC (1999) Why do baleen whales migrate? Mar Mamm Sci 15:1228–1245

Darling J (1991) Humpback whales in Japanese waters. Ogasawara and Okinawa Fluke Identification Catalog 1987–1990. World Wide Fun for Nature Japan or West Coast Research Foundation, Vancouver, BC

Dawbin WH (1966) The seasonal migratory cycle of humpback whales. In: Norris KS (ed) Whales, dolphins, and porpoises. University of California Press, Berkley, CA, p 145–170

Farmer A, Rye R, Landis G, Bern C, Kester C, Ridley I (2003) Tracing the pathways of neotropical migratory shorebirds using stable isotopes: a pilot study. Isotopes Environ Health Stud 39:169–177

Fry B (1981) Natural stable carbon isotope tag traces Texas shrimp migrations. Fish Bull (Wash D C) 79:337–345

Furness RW, Crane JE, Bearhop S, Garthe S and others (2006) Techniques to link individual migration patterns of seabirds with diet specialization, condition and breeding performance. Ardea 94:631–638

Gannes LZ, Obrien DM, delRio CM (1997) Stable isotopes in animal ecology: assumptions, caveats, and a call for more laboratory experiments. Ecology 78:1271–1276

Hobson KA (1999) Tracing origins and migration of wildlife using stable isotopes: a review. Oecologia 120: 314–326

Hobson KA (2006) Establishing migratory connectivity and seasonal interactions using stable isotopes. J Ornithol 147: 52–53

Hobson KA, Clark RG (1992) Assessing avian diets using stable isotopes.II. Factors influencing diet tissue fractionation. Condor 94:189–197

Hobson KA, Schell DM (1998) Stable carbon and nitrogen isotope patterns in baleen from eastern Arctic bowhead whales (*Balaena mysticetus*). Can J Fish Aquat Sci 55: 2601–2607

Hobson KA, Welch HE (1992) Determination of trophic relationships within a high Arctic marine food web using $\delta^{13}C$ and $\delta^{15}N$ analysis. Mar Ecol Prog Ser 84:9–18

Hobson KA, Alisauskas RT, Clark RG (1993) Stable nitrogen isotope enrichment in avian tissues due to fasting and nutritional stress—implications for isotopic analyses of diet. Condor 95:388–394

Hobson KA, Piatt JF, Pitocchelli J (1994) Using stable isotopes to determine seabird trophic relationships. J Anim Ecol 63: 786–798

Kelly JF (2000) Stable isotopes of carbon and nitrogen in the study of avian and mammalian trophic ecology. Can J Zool 78:1–27

Kempster B, Zanette L, Longstaffe FJ, MacDougall-Shackleton SA, Wingfield JC, Clinchy M (2007) Do stable isotopes reflect nutritional stress? Results from a laboratory experiment on song sparrows. Oecologia 151:365–371

Laerm J, Wenzel F, Craddock JE, Weinand D and others (1997) New prey species for northwestern Atlantic humpback whales. Mar Mamm Sci 13:705–711

Lagerquist BA, Mate BR, Ortega-Ortiz JG, Winsor M (2008) Migratory movements and surfacing rates of humpback whales (*Megaptera novaeangliae*) satellite tagged at Socorro Island, Mexico. Mar Mamm Sci 24:815–830

Lockyer C (1981) Growth and energy budgets of large baleen whales from the southern hemisphere. In: Mammals in the seas, Vol 3, Ser 5. FAO Fisheries, Rome, p 379–487

MacAvoy SE, Arneson LS, Bassett E (2006) Correlation of metabolism with tissue carbon and nitrogen turnover rate in small mammals. Oecologia 150:190–201

Mizroch SA, Herman LM, Straley JM, Glockner-Ferrari DA and others (2004) Estimating the adult survival rate of central North Pacific humpback whales (*Megaptera novaeangliae*). J Mammal 85:963–972

Nishiwaki M (1966) Distribution and migration of the larger cetaceans in the North Pacific as shown by Japanese whaling records. In: Norris KS (ed) Whales, dolphins, and porpoises. University of California Press, Berkeley, CA, p 172–191

Perry A, Baker CS, Herman LM (1990) Population characteristics of individually identified humpback whales in the

central and eastern North Pacific: a summary and critique. Rep Int Whal Comm. (Special Issue) 12:307–317

Peterson BJ, Fry B (1987) Stable isotopes in ecosystem studies. Annu Rev Ecol Syst 18:293–320

Podlesak DW, McWilliams SR (2006) Metabolic routing of dietary nutrients in birds: effects of diet quality and macronutrient composition revealed using stable isotopes. Physiol Biochem Zool 79:534–549

Post DM (2002) Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology 83: 703–720

Rau GH, Ainley DG, Bengtson JL, Torres JJ, Hopkins TL (1992) $^{15}N/^{14}N$ and $^{13}C/^{12}C$ in Weddell Sea birds, seals, and fish: implications for diet and trophic structure. Mar Ecol Prog Ser 84:1–8

Rocque DA, Ben-David M, Barry RP, Winker K (2006) Assigning birds to wintering and breeding grounds using stable isotopes: lessons from two feather generations among three intercontinental migrants. J Ornithol 147:395–404

Saupe SM, Schell DM, Griffiths WB (1989) Carbon-isotope ratio gradients in western Arctic zooplankton. Mar Biol 103:427–432

Schell DM, Saupe SM, Haubenstock N (1989a) Bowhead whale (*Balaena mysticetus*) growth and feeding as estimated by $\delta^{13}C$ techniques. Mar Biol 103:433–443

Schell DM, Saupe SM, Haubenstock N (1989b) Natural isotope abundances in bowhead whale (*Baleana mysticetus*) baleen: markers of aging and habitat usage. In: Rundel PW, Ehleringer JR, Nagy KA (eds) Stable isotopes in ecological research. Springer-Verlag, New York, p 260–269

Schell DM, Barnett BA, Vinette KA (1998) Carbon and nitrogen isotope ratios in zooplankton of the Bering, Chukchi, and Beaufort Seas. Mar Ecol Prog Ser 162: 11–23

Steiger GH, Calambokidis J, Sears R, Balcomb KC, Cubbage JC (1991) Movement of humpback whales between California and Costa Rica. Mar Mamm Sci 7:306–310

Tieszen LL, Boutton TW, Tesdahl KG, Slade NA (1983) Fractionation and turnover of stable carbon isotopes in animal tissues—implications for $\delta^{13}C$ analysis of diet. Oecologia 57:32–37

Todd SK (1997) Dietary patterns of humpback whales (*Megaptera novaeangliae*) in the Northwest Atlantic: evidence from ^{13}C and ^{15}N stable isotopes. PhD dissertation, Memorial University of Newfoundland, St. John's

Urbán J, Jaramillo L, Aguayo L, Ladrón de Guevera PP and others (2000) Migratory destinations of humpback whales wintering in the Mexican Pacific. J Cetacean Res Manag 2:101–110

Waite JM, Dahlheim ME, Hobbs RC, Mizroch SA and others (1999) Evidence of a feeding aggregation of humpback whales (*Megaptera novaeangliae*) around Kodiak Island, Alaska. Mar Mamm Sci 15:210–220

Williams CT, Buck CL, Sears J, Kitaysky AS (2007) Effects of nutritional restriction on nitrogen and carbon stable isotopes in growing seabirds. Oecologia 153:11–18

Witteveen BH, Straley JM, von Ziegesar O, Steel D, Baker CS (2004) Abundance and mtDNA differentiation of humpback whales (*Megaptera novaeangliae*) in the Shumagin Islands, Alaska. Can J Zool 82:1352–1359

Witteveen BH, Worthy GAJ, Wynne K, Roth JD (2009) Population structure of North Pacific humpback whales on feeding grounds as shown by stable carbon and nitrogen isotope ratios. Mar Ecol Prog Ser 379:299–310