$\delta^{15}N$ and $\delta^{13}C$ in skin biopsy samples: a note on their applicability for examining the relative trophic level in three rorqual species

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ABSTRACT

Preliminary stable nitrogen and carbon isotope analysis was undertaken to investigate whether the resulting data support current knowledge of diet as obtained by conventional approaches. Blue (*Balaenoptera musculus*), fin (*B. physalus*) and Bryde's (*B. edeni*) whales co-occur temporally and are known to feed in the Gulf of California, Mexico. Isotope measurements were taken from: known prey (three euphausiids and four sardine samples); skin biopsies (two for each whale species); and from faeces (one blue and three fin whale samples). Although the sample size was small, the range of $\delta^{15}N$ values obtained was consistent with prior knowledge of the whales feeding habits, with values increasing in the order: blue ($\bar{x}$ = 12.9), fin ($\bar{x}$ = 15.4) and Bryde's whales ($\bar{x}$ = 15.8). The low value for the blue whale confirms its known stenophagous habit. The closeness of $\delta^{15}N$ values for fin and Bryde's whales coincides with the known icthyophagous habits of the Bryde's whale and the more generalist fin whale which feeds on both fish and zooplankton. The difference in $\delta^{13}C$ values for fin ($\bar{x}$ = 16.0) and Bryde's whales ($\bar{x}$ = 18.1) suggests that although they feed at the same trophic level, they might use different food sources or feeding sites. Results of $\delta^{15}N$ suggest that fin and Bryde's whales share the same relative trophic level, blue whales and juvenile sardines (*S. sagax)* share a lower position, followed by the euphausiid (*Nematocelis difficilis*) and fin whale faeces, and at the lowest level blue whale faeces.

KEYWORDS: FOOD/PREY; BIOPSY SAMPLING; BLUE WHALE; FIN WHALE; BRYDE'S WHALE; ISOTOPES; TECHNIQUES

INTRODUCTION

Knowledge of the feeding habits of cetaceans has been restricted to stomach and faeces-content analysis which provide an estimate of recently consumed prey species. Analysis of stable isotopes using muscle tissue to determine trophic level, has been limited to a few free-ranging species: those captured by native hunters, such as the narwhal, *Monodon monoceros* (Hobson and Welch, 1992) and the bowhead whale, *Balaena mysticetus* (Schell *et al.*, 1989); those caught by whaling nations under scientific permit (Wada *et al.*, 1987); or those caught incidentally during commercial tuna seining (Das *et al.*, 2000). Samples have also been obtained from beached animals (Rau *et al.*, 1983; Ostrom *et al.*, 1993; Abend and Smith, 1995). The use of skin samples obtained from free-ranging cetaceans using biopsy darts (e.g. Lambersten, 1987) was reported to be a good tissue substitute for muscle in stable isotope analysis of North Atlantic humpback whales, *Megaptera novaeangliae*, by Todd *et al.* (1997). This paper presents the results of stable nitrogen and carbon isotope analysis from skin samples of three species of rorquals that co-occur temporally and feed in the Gulf of California in a feasibility study to compare the results from such data with the current understanding of their diet obtained by conventional approaches.

METHODS

Skin samples of blue, fin and Bryde's whales were obtained using a biopsy dart with a 7mm diameter, 40mm long stainless steel core sampler with three internal, inward-facing barbs. The dart was fired from a crossbow at a distance of about 10m from the whale. Using sterilized tweezers, the biopsy was removed from the dart and kept in a sterile glass vial. The dart was sterilized by a blowtorch for about 10 seconds before and after each biopsy to destroy any remaining organic matter and prevent contamination.

Samples of the euphausiid *Nematocelis difficilis* were collected at the sea surface using a glass container. Monterrey sardines (*Sardinops sagax*, $n = 4$) and Japanese sardines (*Etrumeus teres*, $n = 1$) were collected from the stomachs of skipjack tuna. The collected euphausiids were kept alive for some hours before freezing. Faeces of blue and fin whales were also collected at the sea surface. Faecal samples of Bryde's whales could not be collected due to its liquid consistency, typical of icthyophagous cetaceans.

All samples were collected during March and April of 1995 and 1996 in Bahía De La Paz (Fig. 1), except for the blue whale faeces sample, which was collected off the west coast of the Baja California peninsula in the area of San Quintin (30°25'N, 116°10'W) in June 1995. All samples were preserved frozen. Lipids were extracted from the whale skin (previously separated from the blubber) with acetone:hexane (1:1). This non-polar solvent mixture was used primarily to extract non-polar organic compounds (PCBs) following Camacho-Ibar and McEvoy (1996). All freeze-dried samples were ground to a homogeneous fine powder. Samples of 25mg were analysed for $\delta^{15}N$ and $\delta^{13}C$ at the Instituto de Investigaciones Oceanológicas, Universidad Autónoma de Baja California, Mexico. They were run once on a Dual-inlet VG Optima isotope-ratio mass spectrometer interfaced in continuous flow to an elemental analyser NA-1500, Carlo Erba. The National Standards and Technology Institute (NIST) provided ammonium sulphate (IAEA-N_2) used as a standard reference material for calibration of the nitrogen isotopic response of samples ($\delta^{15}N$ *mean* = 20.23 ± 0.17‰; $n = 3$). NBS22-OIL was used as a carbon standard reference material ($\delta^{13}C$ *mean* = –29.75 ± 0.03‰; $n = 6$). Stable nitrogen and carbon isotope ratios are expressed as:

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$$\delta^{15}N \text{ or } \delta^{13}C = [(R_{sample} / R_{standard}) - 1] \times 1{,}000$$

where R is $^{15}N/^{14}N$ for $\delta^{15}N$ and $^{13}N/^{12}C$ for $\delta^{13}C$. The standards for the VG Optima calibration were FISONS® CO_2 (99.8%) with $\delta^{13}C$(‰) referenced to international standard Pee Dee Belemnite = −43.85 and FISONS® N_2 (99.99%) with $\delta^{15}N$ (‰) referenced to atmospheric nitrogen = −0.21.

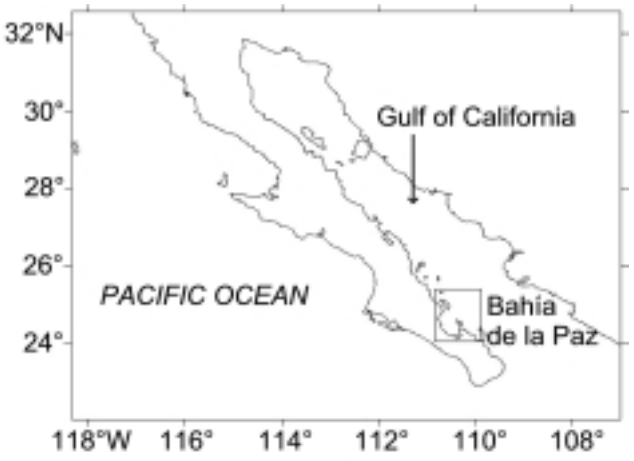

Fig.1. Location of Bahía de La Paz in the Gulf of California.

RESULTS AND DISUSSION

The values observed for all samples were between –22.0‰ and –16.0‰ for $\delta^{13}C$ and 8.6‰ and 15.8‰ for $\delta^{15}N$ (Table 1). An increase in the relative trophic level is shown by the $\delta^{13}C$ *versus* $\delta^{15}N$ relationship (Fig. 2). Blue and fin whale faeces and euphausiids showed the lowest values followed by sardines (*S. sagax* and *E. teres*) and blue whale skin, both with similar values, and fin and Bryde's whale skin tissues giving the highest values. The low sample sizes of isotopic ratios in whale skin ($n = 2$) and prey samples increase uncertainty in the results. However, the increase in relative trophic level appears to discriminate between stenophagous (blue whale) and ichtyophagous or generalist feeders (Bryde's and fin whales) (Fig. 2) and is consistent with the literature on general feeding habits of these rorquals.

Table 1

$\delta^{15}N$ and $\delta^{13}C$ stable isotope values for whales, sardines, euphausiids and whale faeces from Bahía de La Paz.

	n	$\delta^{15}N$ (‰)	SD	$\delta^{13}C$ (‰)	SD
Whales					
B. edeni (skin)	2	15.8	0.6	-18.1	1.5
B. physalus (skin)	2	15.4	1.1	-16.0	0.6
B. musculus (skin)	2	12.9	0.3	-18.2	0.6
Fish					
S. sagax (juvenile)	4	13.3	0.6	-18.5	0.7
E. teres	1	13.9	-	-17.9	-
Euphausiids					
N. difficilis (total)	3	11.0	1.2	-19.5	0.3
Whale faeces (total)					
B. physalus	3	11.1	1.0	-22.0	1.4
B. musculus	1	8.6	-	-18.7	-

The lowest $\delta^{15}N$ value (12.9 ± 0.3‰) from blue whale skin concurs with our knowledge that euphausiids are a significant dietary input for this whale (Nemoto, 1957; Gaskin, 1982). In the southwestern Gulf of California, they are known to be associated with surface swarms of the dominant euphausiid *Nyctiphanes simplex* (Gendron, 1992). They are regularly observed surface feeding on euphausiids and have also been observed defecating (Gendron, 1990).

In contrast, fin and Bryde's whales, whose skin showed higher $\delta^{15}N$ values (15.8 ± 0.6‰ and 15.4 ± 1.1‰), are known not to be restricted plankton feeders but also to feed on small pelagic fish and other prey. Two allopatric forms of Bryde's whales have been reported in South Africa; a fish eater that has a coastal distribution and a plankton feeder distributed offshore (Best, 1977). Both forms have also been recognised in the North Pacific (Omura, 1977). In the Gulf of California, this tropical species is observed year round and is

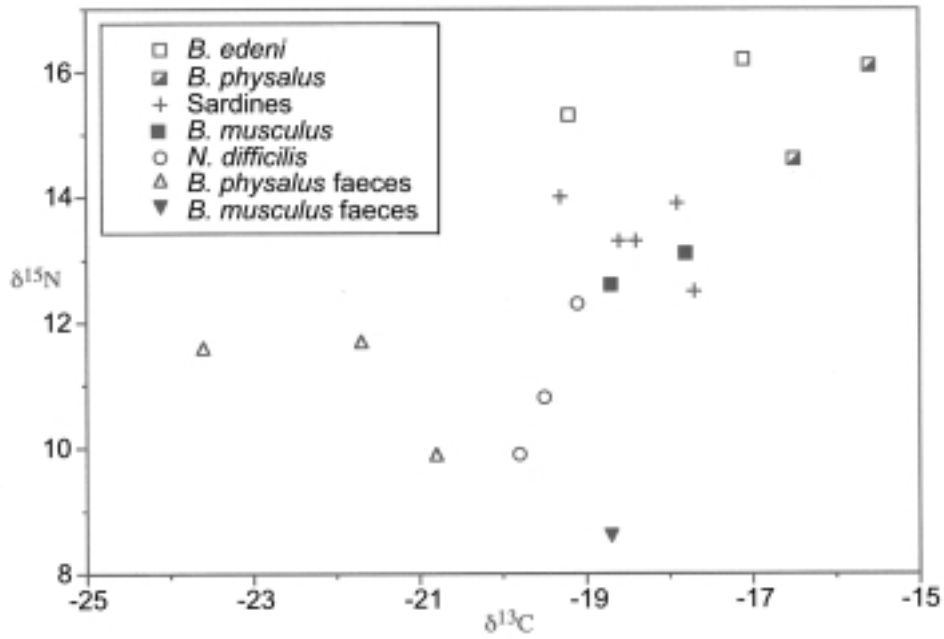

Fig. 2. Distribution of $\delta^{13}C$ and $\delta^{15}N$ values, and their standard deviation (SD), of marine organisms in Bahía de La Paz, Gulf of California. (For sample $n < 4$, all data shown).

mostly seen feeding on small pelagic fish (Tershy, 1992) in the north, but has also been observed feeding on sardines mixed with euphausiids in the southern Gulf of California (Gendron, 1993). In the Northern Hemisphere, the fin whale is recognised as a generalist feeder that varies its diet from zooplankton (copepods and euphausiids) to fish (Nemoto, 1957; Mitchell, 1975; Overholtz and Nicolas, 1979). In the Gulf of California, fin whales are seen throughout the year and are considered a resident population (Tershy *et al.*, 1993). They have been observed feeding principally on euphausiids in the northern Gulf of California (Tershy, 1992) and occasionally on small pelagic fish (Gendron, 1993).

Lipid extraction of humpback whale skin was investigated by Todd *et al.* (1997). From their results it seems that the high standard deviations in $\delta^{13}C$ values for fin and Bryde's whale skin were not caused by lipids. A non-lipid-extracted sample is expected to yield a lower $\delta^{13}C$ than a lipid-extracted sample as shown by Todd *et al.* (1997). The values for fin and Bryde's whales found here are thus probably a reflection of both variation in the species composition of the diet temporal and different feeding locations as described for bowhead whales in Shell *et al.* (1989). We conclude that these two species have distinct foraging locations or different food sources (supported by different $\delta^{13}C$ values) that share a similar relative trophic level (supported by similar $\delta^{15}N$ values).

The suggestion that Bryde's and fin whales feed at a similar trophic level is not in accord with field observations made by Tershy (1992). He reported that Bryde's whales fed mainly on clupeiid fish whereas fin whales were associated with euphausiids. Similarly, finding that fin whales feed at a relatively higher trophic level than blue whales does not coincide with the results obtained from prey items analysed from the faeces of both rorquals sampled in the southern Gulf of California during winter (Del Angel-Rodriguez, 1997). That author reported no difference in feeding habits for the two species which were found to feed mainly on the euphausiid *N. simplex*.

In considering those results, however, it should be recognised that if the fin whale feeds on small fish, such as sardines (as suggested in Fig. 2), the otoliths may either sink rapidly and therefore not be found in the faeces or may be digested (a similar bias could result from the eating of other prey such as squid). The isotopic information presented here represents a time-integrated diet input rather than the instantaneous dietary information obtained from faeces-content analyses and this may explain the different conclusions reached. Although isotopic analysis of faeces cannot be directly linked to prey (isotopic depletion is expected in the nitrogenous portion of the faeces compared to those portions of the diet that are assimilated), the lower value in $\delta^{15}N$ (11.1‰ for fin whale compared to 8.6‰ for blue whale) supports a higher relative trophic component than euphausiids in the diet of the fin whale.

The results based on skin tissue are most likely representative of a relatively short-term picture of the feeding habits of these whales. In a study of epidermal growth in bottlenose dolphins (*Tursiops truncatus*), Hicks *et al.* (1985) found that turnover time for cells to migrate to the surface layer of the skin was 73 days. Assuming the skin turnover time of a subtropical odontocete species (bottlenose dolphins) is similar for baleen whales studied in the Gulf of California, the values here probably reflect an integrated diet consumed two or more months before the biopsies were taken. This suggests that the fin whale might be feeding on a higher relative trophic component such as sardines (Fig. 2) during autumn or early winter, possibly feeding on euphausids later in the winter as found by Del Angel-Rodriguez (1997).

The $\delta^{15}N$ and $\delta^{13}C$ values obtained from the skin are also consistent with the lower values in both stable isotopes found for the different prey analysed (Table 1, Fig. 2). The similar relative trophic level obtained for sardines compared to the blue whale suggests that juvenile *S. sagax* prey on zooplankton rather than phytoplankton. A similar result was found by Monteiro *et al.* (1991) for adult anchovies studied in the southern Benguela Current.

The results for prey are similar to the $\delta^{15}N$ and $\delta^{13}C$ values of euphausiids (11.2 ± 0.5‰, –20.2 ± 0.3‰) and Pacific sardines (12.9 ± 0.1‰, –17.0 ± 0.2‰) from California reported by Sydeman *et al.* (1997). Nitrogen and carbon isotope variations at the base of the food web may be an important factor of isotope composition of upper trophic levels (Burton and Kock, 1999). For instance, lower-latitude plankton food bases tend to be enriched in $\delta^{15}N$ and $\delta^{13}C$ relative to those in high latitude waters (Rau *et al.*, 1991). In this subtropical area, the regenerated nutrients along the west coast of the Gulf of California could promote elevated $\delta^{15}N$ values in top trophic levels compared to those new production-based areas (Aguíñiga, 1999).

The range of $\delta^{13}C$ values for blue whale (–17.6 to –18.8‰) is similar to that obtained from lipid-extracted muscle of a blue whale struck by a ship off California (–17.3, –17.9‰, in Rau *et al.*, 1983). However, such a comparison is probably inappropriate since it has been shown that different tissues of mammals have different $\delta^{13}C$ and $\delta^{15}N$ values (DeNiro and Epstein, 1978; Hobson *et al.*, 1996) and turnover rates (Tieszen *et al.*, 1983) although Todd *et al.* (1997) found no significant difference between isotopic values of skin biopsies and muscle obtained from North Atlantic humpback whales. Todd *et al.* (1997) concluded that use of biopsy samples represents a reliable technique for dietary analysis of cetaceans. However, seasonal changes in prey or feeding locations in other cetaceans might lead to important variations between tissue used and to some extent cause differences in the isotopic results. Similarities (fin and Bryde's whales) and differences (blue whales *versus* the other two species) in isotopic composition cannot be interpreted strictly in terms of food sources but may include a geographical component of unknown importance. For example blue whales (unlike fin and Bryde's whales) are known to migrate out of the Gulf of California to their summering grounds off central California as shown by Calambokidis *et al.* (1990). Their presence in the Gulf of California is reported from late autumn (Vidal *et al.*, 1993) until about May, with a maximum relative abundance in March (Gendron, 1990).

It is possible that the restricted sampling period (March-April) of our study in Bahía De La Paz may have reduced the geographical component and left food source as the most important factor. However, if skin turnover in whales is much longer than two months or transfer from food ingested to skin cells is long, the geographical component in the value of blue whale skin might be important and the result biased by food ingested in other areas.

Although the $\delta^{15}N$ and $\delta^{13}C$ dispersion data are important, the uncertainty in conclusions that can be made from them can be reduced by constructing a trophic-position isotope spectrum to define energy pathways with a large number of samples of whale skin and possible prey species (Monteiro *et al.*, 1991), including sampling in different seasons. A trophic enrichment factor could then be estimated for the Gulf of California. In such circumstances, in

agreement with Todd *et al.* (1997) and Ames *et al.* (1996), we believe that the use of biopsy samples or sloughed skin, will allow a suitable sample size to be obtained so that the feeding habits of free-ranging cetaceans can be evaluated from stable nitrogen and carbon isotope analysis.

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REFERENCES

Abend, A. and Smith, T. 1995. Differences in ratios of stable isotopes of nitrogen in long-finned pilot whales (*Globicephala melas*) in the western and eastern North Atlantic. *ICES J. Mar. Sci.* 52:837-41.

Aguíñiga, S. 1999. Geoquímica de la cuenca estuarina del río Colorado: $\delta^{13}C$, $\delta^{15}N$ y biomarcadores lipídicos en sedimentos superficiales. Doctoral Thesis, Universidad Autónoma de Baja California. 300pp. [In Spanish].

Ames, A.L., Van Vleet, E.S. and Sackett, W.M. 1996. The use of stable carbon isotope analysis for determining the dietary habits of the Florida manatee, *Trichechus manatus latirostris*. *Mar. Mammal Sci.* 12(4):555-63.

Best, P.B. 1977. Two allopatric forms of Bryde's whale off South Africa. *Rep. int. Whal. Commn* (special issue) 1:10-38.

Burton, R.K. and Kock, P.L. 1999. Isotopic tracking of foraging and long-distance migration in northeastern Pacific pinnipeds. *Oecologia* 119:578-85.

Calambokidis, J., Steiger, G.H., Cubbage, J.C., Balcomb, K.C., Ewald, C., Kruse, S., Wells, R. and Sears, R. 1990. Sightings and movements of blue whales off central California 1986-88 from photo-identification of individuals. *Rep. int. Whal. Commn* (special issue) 12:343-8.

Camacho-Ibar, V.F. and McEvoy, J. 1996. Total PCBs in Liverpool Bay sediments. *Mar. Environ. Res.* 41(3):241-63.

Das, K., Lepoint, G., Loizeau, V., Debacker, V., Dauby, P. and Bouqueneau, J.M. 2000. Tuna and dolphin associations in the Northeast Atlantic: Evidence of different ecological niches from stable isotope and heavy metal measurements. *Mar. Poll. Bull.* 40(2):102-9.

Del Angel-Rodriguez, J. 1997. Hábitos alimenticios y distribución espacio-temporal de los rorcuales común (*Balaenoptera physalus*) y azul (*Balaenoptera musculus*) en la Bahía de La Paz, B.C.S., México. MSc Thesis, Centro Interdisciplinario de Ciencias Marinas, La Paz, B.C.S. 82pp.

DeNiro, M.J. and Epstein, D.S. 1978. Influence of diet on the distribution of carbon isotopes in animals. *Geochim. Cosmochim. Acta* 42:495-506.

Gaskin, D.E. 1982. *The Ecology of Whales and Dolphins*. Heinemann, London. 459pp.

Gendron, D. 1990. Relación entre la abundancia de eufáusidos y de ballenas azules (*Balaenoptera musculus*) en el Golfo de California. MSc Thesis, Centro Interdisciplinario de Ciencias Marinas. 64pp. [In Spanish].

Gendron, D. 1992. Population structure of daytime surface swarms of *Nyctiphanes simplex* (Crustacea: Euphausiacea) in the Gulf of California, Mexico. *Mar. Ecol. Prog. Ser.* 87:1-6.

Gendron, D. 1993. Indice de avistamiento y distribución del género *Balaenoptera* en el Golfo de California, México, durante febrero, marzo y abril 1988. *Rev. Inv. Cient.* 1:21-30. [In Spanish].

Hicks, B.D., St Aubin, D.J., Geraci, J.R. and Brown, W.R. 1985. Epidermal growth in the bottlenose dolphin, *Tursiops truncatus*. *J. Invest. Dermatol.* 85(1):60-3.

Hobson, K.A. and Welch, H.E. 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, K.A., Schell, D.M., Renouf, D. and Noseworthy, E. 1996. Stable carbon and nitrogen isotopic fractionation between diet and tissues of captive seals: implications for dietary reconstructions involving marine mammals. *Can. J. Fish. Aquat. Sci.* 53:528-33.

Lambersten, R.H. 1987. A biopsy system for large whales and its use in cytogenetics. *J. Mammal.* 68:443-5.

Mitchell, E.D. 1975. Trophic relationships and competition for food in northwest Atlantic whales. *Proc. Can. Soc. Zool. Ann. Mtg* :123-33.

Monteiro, P.M.S., James, A.G., Sholto-Douglas, A.D. and Field, J.G. 1991. The $\delta^{13}C$ trophic position isotope spectrum as a tool to define and quantify carbon pathways in marine food webs. *Mar. Ecol. Prog. Ser.* 78:33-40.

Nemoto, T. 1957. Foods of baleen whales in the North Pacific. *Sci. Rep. Whales Res. Inst., Tokyo* 12:33-89.

Omura, H. 1977. Review of the occurrence of Bryde's whale in the northwest Pacific. *Rep. int. Whal. Commn* (special issue) 1:88-91.

Ostrom, P.H., Lien, J. and Macko, S.A. 1993. Evaluation of the diet of Sowerby's beaked whale, *Mesoplodon bidens*, based on isotopic comparisons among northwestern Atlantic cetaceans. *Can. J. Zool.* 71(4):858-61.

Overholtz, W.J. and Nicolas, J.R. 1979. Apparent feeding by the fin whale, *Balaenoptera physalus*, and the humpback whale, *Megaptera novaeangliae*, on the American sand lance, *Ammodytes americanus*, in the northwest Atlantic. *Fish. Bull.* 77(1):285-7.

Rau, G.H., Mearns, A.J., Young, D.R., Olson, R.J., Schafer, H.A. and Kaplan, I.R. 1983. $^{13}C/^{12}C$ correlates with trophic level in pelagic food webs. *Ecology* 64(5):1314-8.

Rau, G.H., Hopkins, T.L. and Torres, J.J. 1991. $^{15}N/^{14}N$ and $^{13}C/^{12}C$ in Weddel Sea invertebrates: implications for feeding diversity. *Mar. Ecol. Prog. Ser.* 77:1-6.

Schell, D.M., Saupe, S.M. and Haubenstock, N. 1989. Bowhead (*Balaena mysticetus*) growth and feeding as estimated by $\delta^{13}C$ techniques. *Mar. Biol.* 103(4):433-43.

Sydeman, W.J., Hobson, K.A., Pyle, P. and McLaren, E.B. 1997. Trophic relationships among seabirds in central California: combines stable isotope and conventional dietary approach. *Condor* 99:327-36.

Tershy, B.R. 1992. Body size, diet, habitat use, and social behavior of *Balaenoptera* whales in the Gulf of California. *J. Mammal.* 73(3):477-86.

Tershy, B.R., Urbán-Ramírez, J., Breese, D., Rojas-Bracho, L. and Findley, L.T. 1993. Are fin whales resident to the Gulf of California? *Rev. Inv. Cient.* 1:69-72.

Tieszen, L.L., Boutton, T.W., Tesdahl, K.G. and Slade, N.A. 1983. Fractionation and turnover of stable carbon isotopes in animal tissues: implications for $\delta^{13}C$ analysis of diet. *Oecologia* 57:32-7.

Todd, S., Ostrom, P., Lien, J. and Abrajano, J. 1997. Use of biopsy samples of humpback whale (*Megaptera novaeangliae*) skin for stable isotope $\delta^{13}C$ determination. *J. Northwest Atl. Fish. Sci.* 22:71-6.

Vidal, O., Findley, L.T. and Leatherwood, S. 1993. Annotated checklist of the marine mammals of the Gulf of California. *Proc. San Diego Soc. Nat. Hist. (USA)* 28:1-11.

Wada, E., Terazaki, M., Kabaya, Y. and Nemoto, T. 1987. $\delta^{15}N$ and $\delta^{13}C$ abundance in the Antarctic Ocean with emphasis on the biogeochemical structure of the food web. *Deep-Sea Res.* 5/6:829-41.