DIETARY PATTERNS OF HUMPBACK WHALES (*Megaptera novaeangliae*) IN THE NORTHWEST ATLANTIC: EVIDENCE FROM ^{13}C AND ^{15}N STABLE ISOTOPES

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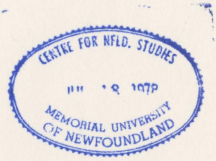
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Dietary Patterns of Humpback Whales (*Megaptera novaeangliae*) in the Northwest Atlantic: Evidence From ^{13}C and ^{15}N Stable Isotopes

by

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Abstract

Previous dietary assessments of the northwest Atlantic humpback whale (*Megaptera novaeangliae*), mainly based on analysis of gut contents, may now be outdated because of recent substantial biological and oceanographic changes in the northwest Atlantic ecosystem. Within ecological studies, newer techniques are being developed that examine diet non-lethally. Stable isotope analysis (SIA), for example, utilizes prey-specific signatures preserved in the tissues of a consumer, and holds several advantages over traditional techniques of prey determination, including examination of longitudinal variation in diet. This thesis used SIA to assess diet in the Newfoundland and Labrador feeding substock of humpback whales. Two stable-isotope indicators—$\delta^{13}C$ and $\delta^{15}N$—were used to interpret types of diet and trophic feeding level.

Previous SIA studies used muscle tissue from dead, stranded cetaceans. However, recent advances in biopsy technique have provided a method to collect skin and blubber samples from free-ranging animals in a non-lethal manner. This study calibrated the two techniques using tissues from dead and live animals. Analyses demonstrated that isotopic ratios estimated for either tissue were statistically indistinguishable. Levels of $\delta^{15}N$ and $\delta^{13}C$ were measured with high accuracy and precision (± 0.2‰).

A second part of this study is based on a collection of biopsied samples (n = 130) taken in 1988–1994 from humpback whales on their feeding grounds in the northwest Atlantic. Isotopic ratios varied among regions, probably because of large scale oceanographic differences affecting phytoplankton isotopic composition, and because of differences in prey distribution. Isotopic ratios did not vary by sex.

Examination of monthly data, correlated with prey availability, provided a first estimation of the integration time for skin tissue (~7–14 days). These data support the

hypothesis that skin is a short-turnover tissue, and its isotopic composition reflects short-term diet (< 30 days). Large isotopic variations within and between years are attributed to differences in diet and large-scale oceanographic changes. Possible decreases in primary productivity in the early 1990s due to the North Atlantic Oscillation were correlated with increases in $\delta^{15}N$ values. This finding suggested changes in humpback diet as prey species changed in distribution, availability and abundance in response to colder, less saline waters.

In 1994, an opportunistic collection of biopsies from finback whales (*Balaenoptera physalus*) feeding in the same area as humpback whales confirmed that finback whales fed at lower trophic levels. Comparison of the isotopic values for humpback whales with other mysticete species suggests that the humpbacks whale is piscivorous.

In summary, biopsy samples may provide useful indicators of diet using stable-isotope methods. Stable-isotopic analysis provides researchers with a relatively non-intrusive and non-lethal method that, if used in conjunction with other techniques, can provide an accurate assessment of diet.

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Table of Contents

List of Tables

Table

List of Figures

Figure

1. Introduction

1.1 Outline of life history and population biology of the humpback whale

The humpback whale (*Megaptera novaeangliae*) is a medium-sized balaenopterid found in most of the world's oceans, and is distributed in several distinct populations (Mackintosh 1965; Winn and Reichley 1985; Whitehead 1987). It is a seasonal migrant (Mackintosh 1965; Dawbin 1966), wintering at low latitudes to mate and reproduce (Mackintosh 1965; Lockyer 1984; Winn and Reichley 1985). During the spring and summer, humpbacks migrate to high latitudes to feed in productive temperate, boreal and low-Arctic waters (Mackintosh 1965; Dawbin 1966).

The largest population of humpback whale resides in the Northwest Atlantic, with ~7700 animals (95% confidence interval, 2900–8100; Katona and Beard 1990; Palsbøll *et al.* 1997). This population winters in the Caribbean, primarily the Silver and Navidad Banks of the West Indies (Mattila and Clapham 1989; Mattila *et al.* 1989, 1994). During its northward migration in the spring (Clapham and Mattila 1988, 1990), animals separate into substocks that utilize discrete feeding areas, and display year-to-year site fidelity (Clapham *et al.* 1993). These subregions include the Gulf of Maine, West Greenland, Iceland, and the Gulf of St. Lawrence (Edds and Macfarlane 1987; Katona and Beard 1990). The largest substock (≥ 3000 animals) migrates to the low-Arctic waters of Newfoundland and Labrador to feed from June to September (Whitehead 1987; Katona and Beard 1990).

1.2 Foraging behaviour and diet of the humpback whale

For humpbacks, little feeding occurs during the winter (Dawbin 1966; Lockyer 1981; but see Baraff *et al.* 1991; Laerm *et al.* 1997). Feeding may occur during the migration to feeding

subregions, and is common during the summer in the high-latitude phase of the humpback's migratory cycle (Dawbin 1966; Lockyer 1981). Lockyer (1981) demonstrates that during ~5 months of feeding, humpbacks may double their body weight; thus the summer feeding period is clearly important.

Humpback whales in boreal Alaskan and Southern Hemisphere waters are mainly planktivorous—feeding on small crustaceans collectively referred to as krill (Nemoto 1959; 1970; Lockyer 1981). In the Northwest Atlantic, humpbacks are classified as mainly piscivorous (Nemoto 1959, 1970), feeding on small schooling fish—such as sandlance (*Ammodytes americanus*) in the Gulf of Maine, and capelin (*Mallotus villosus*), herring (*Clupea harengus*), and squid species in low-Arctic Newfoundland waters (Sergeant 1966; Mitchell 1973, 1974; Overholtz and Nicolas 1979; Kawamura 1980; Bredin 1986; Piatt *et al.* 1989; Payne *et al.* 1990). Consumption of small crustaceans (e.g., *Meganyctiphanes norvegica*, *Calanus* spp.), has also been demonstrated in humpbacks killed during whaling in eastern Canada (Nemoto 1959; Sergeant 1966; Mitchell 1974; Kawamura 1980). Geographic differences in dominant prey type likely reflect spatial variation in prey availability (Overholtz and Nicolas 1979; Hain *et al.* 1982).

Whitehead and Carscadden (1985) correlated yearly counts of Northwest Atlantic balaenopterids with capelin year-class strengths. They found that humpback and finback (*Balaenoptera physalus*) whale numbers were predicted by abundance of 2–3 year old capelin, whereas minke whale (*B. acutorostrata*) numbers correlated with abundance of 1-2 year old capelin. These data suggest that, for balaenopterids, specialization may occur even within one prey species. Furthermore, Piatt and Methven (1992) demonstrated that the onset of foraging behaviour for the three balaenopterids was related to differences in capelin school density. Minke whales foraged on low densities of capelin, humpbacks intermediate densities, and finbacks high densities (~3, 7 and 9 schools.km^{-1}, respectively; Dolphin 1987; Piatt and Methven 1992).

1.3 Determination of historic and present-day diet in humpback whales

Traditional methods of assessing diet and feeding behaviour include analysis of gut contents, scat collection, observation of feeding behaviours, and correlation of prey and consumer distributions. Analysis of contents is based on the retention of identifiable prey parts in the gut (Pierce and Boyle 1991). Owing to differential digestion, these results can be biased such that hard parts that are difficult to digest (chitinous shells, calcareous otoliths, squid beaks, etc.) are over-represented, and easily digested tissues are absent or under-represented (Gearing 1991; Harvey and Antonelis 1994; Gould *et al.* 1997). Thus, analysis of gut contents is biased towards the most recent, partially undigested diet, or dietary content that is relatively indigestible. Also, such analyses are generally performed *post mortem*. Stomach lavage and regurgitation can be used to examine stomach contents non-lethally (Harvey and Antonelis 1994), although such techniques are virtually unusable when applied to large, fully aquatic marine mammals .

Analysis of gut contents remains the primary method for assessing marine mammal diets (Pierce and Boyle 1991). For humpback whales, access to such samples has relied on commercial whaling (Nemoto 1959; Sergeant 1966; Nemoto 1970; Mitchell 1973, 1974; Kawamura 1980). Humpbacks in the Northwest Atlantic population have been protected since 1955 by the International Whaling Commission, with the exception of scientific collections from 1966 to 1971 (Sergeant 1966; Mitchell 1973, 1974; Kawamura 1980; Mitchell and Reeves 1983; Whitehead 1987). Apart from occasional opportunistic dissections of stranded or net-entrapped animals found in Newfoundland waters (which showed humpback stomachs to contain capelin and sand lance; J. Lien unpub. data), current gut-content data for the Northwest Atlantic humpback are not available, essentially a result of the 1971 whaling moratorium.

Given the almost ubiquitous reliance on data from analysis of gut contents to determine diet of humpback whales, inferences on long-term annual changes in diet for the Northwest Atlantic population of humpback whales are limited. Such inferences can be important, because in recent

decades, several important biological and oceanographic events have occurred in the Northwest Atlantic. First, since the cessation of whaling in 1971, the humpback whale population in this region has increased (Sergeant 1966; Whitehead 1987; Palsbøll *et al.* 1997). Second, during the 1990s, North Atlantic waters became colder and less saline (Myers and Akenhead 1988; Montevecchi and Myers 1992; Drinkwater and Trites 1993; Colbourne 1994; Drinkwater *et al.* 1994; Mann and Drinkwater 1994; Drinkwater 1997), as a function of the North Atlantic Oscillation (NAO; Myers and Akenhead 1988; Mann and Drinkwater 1994). Associated increased periods and areas of ice cover have also been noted (Mann and Drinkwater 1994; Drinkwater 1997). Such changes might influence local productivity and distribution of ecologically important species and thus affect prey availability to humpbacks (Gomes *et al.* 1995; Narayanan *et al.* 1995; Montevecchi and Myers 1996). Finally, as a result of overfishing, extreme declines in populations of most commercial groundfish species of fish have occurred (Hutchings and Myers 1995; Montevecchi and Myers 1996; Myers *et al.* 1996; Myers *et al.* in press). Thus previous assessments of humpback diet based upon analysis of gut contents may be outdated.

Lack of a longitudinal aspect in *post mortem* gut-content analyses prohibits determination of individual variability in prey selection. A first estimation of such variability can be made through one-time sampling of different individuals within a feeding aggregation at spatial or temporal intervals of interest, assuming that prey selection is constant within a sampled aggregation. For example, Nemoto (1959) characterized annual dietary changes in various mysticetes from 13 years of whaling records. This study could not, however, provide longitudinal information on variability in diet selection by a specific individual with respect to time and location.

A more useful study of the diet of an individual's would involve non-lethal, repeated determinations. For example, collection of fecal material from the consumer can aid in prey identification (Murie 1987; Prime and Hammond 1987, 1990; Cottrell *et al.* 1996). Scat-

collection techniques can also be adapted to investigate assimilation efficiency, and thus indicate, to an extent, nutritional dependence on a particular dietary item (Fadely *et al.* 1990). However, scat collection has biases similar to analyses of gut contents, and collection of feces from a fully aquatic wild animal is similarly impractical.

An alternative non-invasive technique is to observe feeding bouts upon identified prey items, or to observe assumed associations between the subject animal and presumed prey items. With respect to the former, observations of feeding balaenopterids are generally limited to the surface, although SONAR has been used to a limited extent in detection of underwater feeding (Overholtz and Nicolas 1979; Watkins and Schevill 1979; Hays *et al.* 1985; Bredin 1986; Dolphin 1987; Edds and Macfarlane 1987; Hoelzel *et al.* 1989; Piatt *et al.* 1989; Tershy 1992; Weinrich *et al.* 1992). With respect to the latter, direct or indirect associations may be assessed. Direct associations involve the matching of prey and balaenopterid distributions (Whitehead *et al.* 1980; Bryant *et al.* 1981; Whitehead and Carscadden 1985; Whitehead and Glass 1985; Kenney and Winn 1986; Piatt *et al.* 1989; Payne *et al.* 1990). Indirect associations may utilize observations of other species (e.g., gulls) feeding on prey items presumed to be common to the consumer of interest and the observed species (Perkins and Whitehead 1977).

A salient problem in observational studies is that it is often difficult to confirm prey species taken (Overholtz and Nicolas 1979; Watkins and Schevill 1979; Hays *et al.* 1985). Such techniques are more useful in determining distributional relationships rather than diet selection *per se*. This is because feeding by the subject of interest may never be observed directly, and because the determination of the presumed prey is usually based on the examination of gut contents taken from other animals (Piatt *et al.* 1989; Piatt and Methven 1992; Marques 1997). Thus, whereas observation of feeding is a non-invasive technique that permits resampling, when used exclusively it cannot provide an accurate assessment of diet.

In summary, traditional techniques of dietary study in humpback whales have serious practical limitations. Few data are available on changes in diet since the regrowth of the

Northwest Atlantic population of humpbacks, or of changes caused by recent redistributions or depletions of either potential prey stocks or fish species that feed on those prey stocks. Analysis of gut contents provides only a record of the most recent diet, presuming undigested quantities of prey species remain in the gut to be identified. Methods of diet determination that use direct and indirect observations of feeding bouts can only assess presumed feeding activity at the surface.

1.4 An introduction to stable-isotopic analysis (SIA)

Several methods have been developed that monitor traceable chemical components introduced through diet (Huntley *et al.* 1987; Worthy in press). Stable-isotopic analysis (SIA), of a whole organism (or its constituent tissues) is a biogeochemical technique that has been adapted to provide information on diet, trophic relationships, and the base of nutritional support to a consumer (McConnaughey and McRoy 1979a, 1979b; Minigawa and Wada 1984; Wada *et al.* 1987; Harrigan *et al.* 1989; Hobson and Welch 1992; Hobson 1993; Ostrom and Fry 1993; Hobson *et al.* 1994, 1997). Using naturally occurring tracers, the technique provides complementary and unique data relative to traditional methods of dietary assessment (Peterson and Fry 1987; Gearing 1991; Worthy in press). For example, whereas analysis of gut contents provides information on the most recent meals, the stable-isotopic ratio of an organism reflects material that has been assimilated over longer periods of time, depending on the metabolic turnover time of the tissue measured (Tieszen *et al.* 1983).

In biological research the common stable-isotopes monitored include ^{2}H, ^{13}C, ^{15}N, ^{18}O, and ^{34}S (Ehleringer and Rundel 1989). However, many ecological studies limit their examination to ^{13}C and ^{15}N (Peterson and Fry 1987). The abundance of a stable isotope in a sample of interest is reported as a ratio in comparison with its more abundant form. This ratio (R_{sample}) is compared to a reference ratio ($R_{reference}$) and calculated as a delta (δ) value (in units per mil):

EQUATION 1:

$$\delta^{y}X\ (‰) = \left(\frac{R_{sample}}{R_{reference}} - 1\right) \times 10^{3}, \qquad \text{where } X = \text{stable isotope of interest}$$

As this thesis examines the behaviour of ^{13}C and ^{15}N (^{y}X in equation 1), R in the above equation represents the ratios $^{13}C/^{12}C$ or $^{15}N/^{14}N$, and abundance of either isotope is ultimately expressed as $\delta^{13}C$ and $\delta^{15}N$, respectively. Reference ratios used are calibrated either to PeeDee belemnite (PDB) in the case of $\delta^{13}C$, or atmospheric molecular nitrogen in the case of $\delta^{15}N$ (Ehleringer and Rundel 1989). Equation 1 demonstrates that increases in the amount of ^{15}N or ^{13}C in a sample relative to a reference ratio would be reflected by increases in $\delta^{15}N$ and $\delta^{13}C$ respectively. In the marine biosphere, $\delta^{13}C$ levels tend to be negative as most marine organisms are depleted in ^{13}C in comparison to PDB. Alternatively, $\delta^{15}N$ values tend to be positive because of an enrichment in ^{15}N in the organism relative to atmospheric nitrogen (Ehleringer and Rundel 1989).

In ecological studies, the principle use of SIA is a comparison of stable-isotopic values of the diet to that of the consumer. In general, the isotopic composition of the diet and consumer are similar, or differ by a consistent amount (DeNiro and Epstein 1978; 1981; Peterson and Fry 1987). For example, the ~15–20‰ difference in the $\delta^{13}C$ values of C_3 and C_4 plants is also seen in consumers fed exclusively on C_3 or C_4 vegetation (Park and Epstein 1960; Smith and Epstein 1971; DeNiro and Epstein 1978; Fry *et al.* 1978; Boutton *et al.* 1988; Ehleringer and Rundel 1989; Fogel and Cifuentes 1993).

Small differences in isotopic values between the consumer and its diet are a consequence of fractionation or isotopic discrimination during metabolism. For example, the difference in $\delta^{13}C$ between a consumer and its food is small, generally ±1‰ or less (DeNiro and Epstein 1978), which generally results in changes of no more than 1‰ per trophic level (Fry *et al.* 1984; Fry

and Sherr 1984; Ostrom and Fry 1993). In contrast, an enrichment of ~3‰□in $\delta^{15}N$ is observed between a consumer and its diet (DeNiro and Epstein 1981; Minigawa and Wada 1984; Wada *et al.* 1987; Ostrom and Fry 1993). These enrichments occur between each trophic level in a food web (Schoeninger and DeNiro 1984; Fry 1988; Ostrom and Fry 1993). Thus, nitrogen stable-isotopic values provide a robust indicator of trophic position of a consumer because of the larger fractionation associated with trophic enrichment. Trophic level of a consumer (TL) can be quantified by:

EQUATION 2:

$$TL = 1 + \left(\frac{\delta_{cons.} - \delta_{prim.prod.}}{f} \right)$$

where the trophic fractionation constant f per trophic level for $\delta^{15}N$ is assumed to be 3‰, $\delta_{cons.}$ is the $\delta^{15}N$ value for the consumer, and $\delta_{prim.prod}$ is the $\delta^{15}N$ value for the primary producer at the base of the food chain.

The principle of stepwise enrichment per trophic level in isotopic ratios has been used in studies of several low-Arctic/Boreal (Hobson and Welch 1992; Rau *et al.* 1992), and Northwest Atlantic (Dickson 1987; Wada *et al.* 1987; Fry 1988; Hobson and Montevecchi 1991; Ostrom *et al.* 1993) food webs. Occasionally, studies investigating trophic relationships use both $\delta^{13}C$ and $\delta^{15}N$ variables together to indicate feeding at different trophic levels (Hobson 1993; Hobson *et al.* 1994). This multivariate approach to separate discrete groups (in this case, species) can be more successful in detecting differences than by relying on one isotope variable alone.

Levels of $\delta^{13}C$ on their own are less reliable indicators of trophic level because of the associated lower value of f. Instead, $\delta^{13}C$ levels are often used to imply source of nutrient uptake, because of distinguishable isotopic differences between primary producers from

different ecosystems (Stephenson *et al.* 1986; Hobson 1987; Dunton *et al.* 1989; Ramsay and Hobson 1991; Thompson *et al.* 1995; Hobson *et al.* 1997b).

1.5 Factors affecting $\delta^{15}N$ and $\delta^{13}C$ signatures in consumers

Gearing (1991) stated that $\delta^{13}C$ values in a consumer are the result of: a) assimilated diet; b) differences in the biochemical fraction and tissue type measured; and c) other interacting factors such as size, age, sex and health of the consumer. Nitrogen isotopic-values are affected by similar factors (Gannes *et al.* 1997). DeNiro and Epstein (1981) and Minigawa and Wada (1984) demonstrated that consumer $\delta^{15}N$ values are a function of dietary isotopic composition. Stable-nitrogen values also vary as a function of tissue type (Tieszen *et al.* 1983; Hobson and Clark 1992b; Gannes *et al.* 1997). Hobson *et al.* (1993) demonstrated the effects of metabolic stress on fractionation values. Fasting animals exhibit tissues enriched in ^{15}N and ^{13}C relative to non-fasting animals, because the heavier isotopes are discriminated against in metabolic processes. Thus, nutritionally stressed animals display higher values of $\delta^{13}C$ and $\delta^{15}N$ (Hobson *et al.* 1993; Gannes *et al.* 1997).

The influence of diet in the isotopic composition of a consumer may differ between laboratory-raised and wild animals. In most laboratory experiments animals are fed the same diet over a long period of time (e.g., DeNiro and Epstein 1978, 1981; Minigawa and Wada 1984). However, consumers in the wild do not feed exclusively on one dietary item, nor may particular dietary items be continuously available. Isotope ratios in the consumer represent the assimilated portion of a diet, comprised of materials with isotopic ratios from individual prey items weighted by their proportion in the overall diet. By using standard mixing equations, previous studies have predicted end-consumer isotopic compositions based on a knowledge of fractionation, prey isotopic composition and weights for two-prey (Hobson 1990; Hobson and Sealy 1991; Hobson 1993) and three-prey (Harrigan *et al.* 1989) systems. Because of specific

metabolic routing of dietary components, for example either in energy-releasing catabolism or for tissue synthesis, knowledge of the ultimate destination for assimilated prey components can be critical in such calculations (Gannes *et al.* 1997). Also, the relative proportion of individual dietary items is usually not constant, and this results in variation in the isotopic signature of the consumer during periods of diet change.

Variations in isotopic values across tissues are caused by differences in biochemical composition and metabolic activity (turnover) between tissues. For example, variability in the percentage lipid fraction of a tissue can lead to $\delta^{13}C$ values biased towards ^{12}C, because unique metabolic pathways associated with lipid metabolism result in low $\delta^{13}C$ values for lipids relative to other tissues (DeNiro and Epstein 1977). Fractionation differences between a high-lipid and low-lipid content tissue from the same animal can be ~10‰ (Appendix B), an amount that could mask fractionation differences caused by trophic level (for $\delta^{13}C$, ~1‰). Mathematical normalization of isotopic ratios can counter the bias of ^{13}C depleted lipids (McConnaughey and McRoy 1979b; Fry *et al.* 1984; Rau *et al.* 1992). An alternative strategy is to chemically remove the lipid fraction from tissues prior to SIA.

Finally, it is important to acknowledge the time it takes for prey isotopic-levels to be integrated into the consumer. Isotopic composition of the sampled consumer tissue represents an integral of dietary values assimilated and averaged across the metabolic turnover (or half-life) of that tissue (Tieszen *et al.* 1983). Several studies examine turnover rate of various tissues using stable isotopes (Jones *et al.* 1981; Fry and Arnold 1982; Tieszen *et al.* 1983; Boutton *et al.* 1988; Hobson and Clark 1992a), with the general finding that isotopic ratios taken from long-turnover, metabolically slow tissues, represent long-term dietary averages. Metabolically inert tissues have isotopic ratios equivalent to the diet taken at the time of initial tissue deposition, with little or no subsequent change (Schell *et al.* 1989a). Metabolically active (or short-turnover) tissues give short-term dietary averages. Thus, although the examination of teeth, or the scales of fish might provide a dietary average in the order of years, isotopic ratios

measured in muscle tissues from the same animal might provide information concerning diet assimilated within the previous month (Tieszen *et al.* 1983; Wainwright *et al.* 1993; Hobson *et al.* 1994; Thewissen *et al.* 1996). Sources such as blood or milk have extremely brief turnover times (cf. 10 and 0.9 days; Boutton *et al.* 1988; Hobson and Clark 1992a). Animals exhibiting high growth rates (for example, juveniles) show relatively brief turnover rates because of elevated metabolic rates (Fry and Arnold 1982).

1.6 Present knowledge of stable isotopes in marine mammals

Most stable-isotopic studies performed on marine mammals to date have used dead animals as a source of tissue for analysis. Such studies include pinnipeds (Schoeninger and DeNiro 1984; Hobson and Welch 1992; Rau *et al.* 1992; Hobson *et al.* 1997b), odontocetes (Schoeninger and DeNiro 1984; Nelson *et al.* 1991; Ostrom *et al.* 1993; Abend and Smith 1995, 1997), mysticetes (Schoeninger and DeNiro 1984; Schell *et al.* 1989a, 1989b; Withrow *et al.* 1992; Ostrom *et al.* 1993; Schell and Saupe 1993), polar bears (*Ursus maritimus*; Ramsay and Hobson 1991; Hobson and Welch 1992), and sea otters (*Enhydra lutris*; Schoeninger and DeNiro 1984). To date, few studies have utilized SIA for study of marine mammals using material taken non-lethally from live animals. These include examinations of: the West Indian manatee, *Trichechus manatus* (Ames *et al.* 1996); humpback and finback whales (Borobia *et al.* 1995); captive harp (*Phoca groenlandica*), harbour (*P. vitulina*) and ring (*P. hispida*) seals (Hobson *et al.* 1996); polar bears (Hobson and Stirling 1997); and an opportunistic value taken from a blue whale (*B. musculus*; Rau *et al.* 1983). A summary of current isotopic data reported for marine mammals in given in Appendix A.

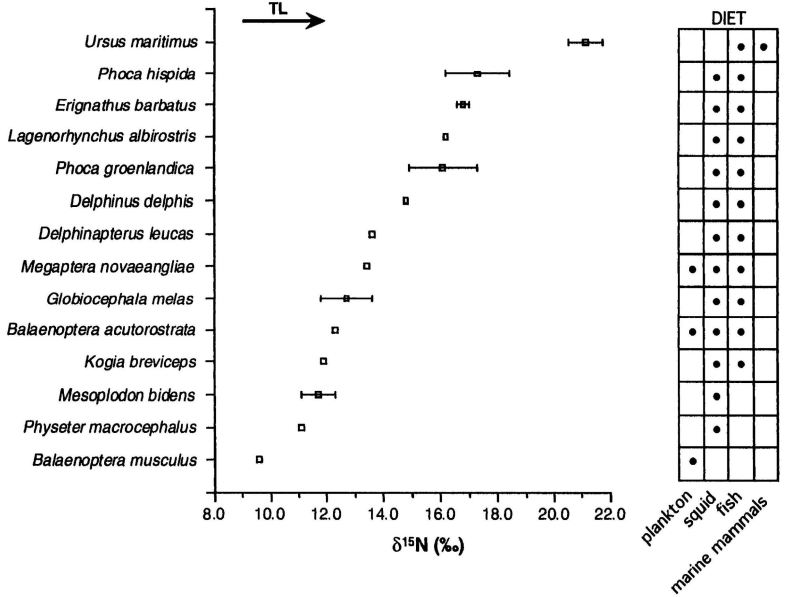
In general, SIA of marine mammals has been used to investigate diet and trophic level. Several studies examine trophic level and diet of marine mammals in the North Atlantic (Abend 1993; Ostrom *et al.* 1993; Abend and Smith 1995; Borobia *et al.* 1995; Abend and Smith

1997). When such data are grouped, trophic differences are easily distinguished using levels of $\delta^{15}N$ (Figure 1.1). For example, planktivores feeding at lower trophic levels are separated from teuthophagous (squid-eating) cetaceans, piscivorous cetaceans and pinnipeds, and predators of other marine mammals. Schoeninger and DeNiro (1984) demonstrated a similar hierarchy in $\delta^{15}N$ levels as a function of trophic level for a broad range of marine mammal species, mostly sampled in the Pacific Ocean.

Several studies have used SIA to determine the trophic level of seals in marine ecosystems (Rau *et al.* 1992; Hobson *et al.* 1996, 1997b). Such analyses are particularly relevant in the Northwest Atlantic ecosystem, where SIA has been used to clarify fishery-seal interactions. For example, harp seal predation on cod (*Gadus morhua*) has, in the past, been cited as a potentially limiting mechanism in the recovery of northern cod stocks (Harris 1990; Anonymous 1997), despite the lack of conclusive evidence. Stable-isotopic methods provided a means to actively monitor diet of harp seals (Hobson *et al.* 1996). Isotopic values obtained from the muscle of harp seals suggest that, similar to sympatric ring and bearded (*Erignathus barbatus*) seals, the diet of harp seals consists primarily of small pelagic fish—perhaps capelin and Arctic cod (*Boreogadus saida*; W. Montevecchi and K. Hobson unpub. data), but not northern cod. Importantly, because SIA can measure assimilated long-term diet, the technique also demonstrated that harp seals might take higher proportions of planktonic crustaceans than previously thought. These prey species may have been previously underestimated by stomach content analysis because such dietary components would be rapidly digested (W. Montevecchi and K. Hobson unpub. data).

In studies of trophic level assessment, estimates of trophic hierarchy are based on $\delta^{15}N$ values, and the observation that the ^{15}N fractionation associated with each trophic level (f_N) is 3‰. This fractionation estimate is strongly supported by many studies (Minigawa and Wada 1984; Schoeninger and DeNiro 1984; Owens 1987; Ostrom and Fry 1993; Ostrom *et al.* 1993; Michener and Schell 1994). However, Abend (1993) calculated f_N to be 2.1‰ for captive long-

Figure 1.1. Mean $\delta^{15}N$ values (± *SD* where available) for various marine mammals found in the North Atlantic. A table on the right of the graph broadly categorizes the diet of each species as either plankton, squid, fish or marine mammals. Ordering by trophic level of diet demonstrates the trophic enrichment of nitrogen-isotopic values. Marine mammals feeding exclusively on plankton have the lowest $\delta^{15}N$ values. Polar bears that feed upon other marine mammals have the highest $\delta^{15}N$ values. The vector labeled TL indicates the ^{15}N enrichment associated with one trophic level, assumed to be 3‰ (see text). Data come from Hobson and Welch (1992), W. Montevecchi and K. Hobson (unpub. data), Ostrom *et al.* (1993), and Abend and Smith (1997).


TL
Ursus maritimus
Phoca hispida
Erignathus barbatus
Lagenorhynchus albirostris
Phoca groenlandica
Delphinus delphis
Delphinapterus leucas
Megaptera novaeangliae
Globiocephala melas
Balaenoptera acutorostrata
Kogia breviceps
Mesoplodon bidens
Physeter macrocephalus
Balaenoptera musculus
8.0
10.0
12.0
14.0
16.0
18.0
20.0
22.0
δ15N (‰)
DIET
plankton
squid
fish
marine mammals

finned pilot whales (*Globicephala melas*) fed a constant diet. This value is likely due to the use of an unusual tissue source from the consumer (blood). Similar low enrichment levels were reported for blood by Hobson *et al.* (1996) in captive harp seals. Abend and Smith (1997) also suggest lower enrichment constants (1.1‰ and 1.7‰ for muscle and skin, respectively) for long-finned pilot whales caught in fishing gear. These values of f_N are based an assumed diet. However, this diet may not reflect the prey actually taken by the animals. Thus, pending further evidence, this thesis assumes f_N to be 3‰, although it is acknowledged that this figure may vary according to the biochemical composition of the tissue examined.

One benefit of SIA is its ability to measure dietary composition non-invasively, assuming the tissue can be accessed without consequence to the animal. This approach was taken by Borobia *et al.* (1995), who presented $\delta^{13}C$ values for humpback and finback whales in the Gulf of St. Lawrence derived from the lipid component of blubber samples taken by non-lethal biopsy. Borobia *et al.* (1995) show a significant difference between the two species in $\delta^{13}C$, suggesting that humpbacks in the Gulf of St. Lawrence feed at a slightly lower trophic level than finbacks.

Because of its non-invasive aspect, SIA studies can be adapted to examine the same individuals longitudinally. Ames *et al.* (1996) collected sloughed skin from captive manatees to correlate non-lipid-extracted $\delta^{13}C$ values with known dietary values. Killingley (1980) documented changes in location of gray whales (*Eschrichtius robustus*), using $\delta^{18}O$ values taken from the shell of an epizoic parasite (^{18}O is known to be sensitive to changes in environmental temperature; Ehleringer and Rundel 1989). Values of $\delta^{18}O$ have also been used to determine reliance on freshwater or marine systems in prehistoric cetaceans, by sampling fossilized teeth (Thewissen *et al.* 1996).

The technique of repeated sampling of the same individual was also used by Schell *et al.* (1989a, 1989b, 1993) to quantify migrations of bowhead whales (*Balaena mysticetus*) between the Bering, Chukchi and Beaufort Seas, by sampling baleen plates taken from hunted whales.

Baleen plates are thought to grow in a series of annual layers; once deposited, such keratinous material is essentially metabolically inert. Because the Beaufort and Bering Seas have prey bases of differing isotopic compositions, the $\delta^{13}C$ value of each baleen layer is thought to record an annual migration cycle. The authors used this technique to age young bowhead whales, by counting the number of detectable oscillations within a baleen plate (Schell *et al.* 1989a, 1989b; see also Withrow *et al.* 1992; Schell and Saupe 1993).

In summary, SIA methods can provide long-term dietary information for marine mammals beyond that obtainable by stomach content analysis and other traditional methods of dietary analysis (Schell *et al.* 1989a; Gearing 1991; Hobson *et al.* 1996). Diet composition can be quantified assuming detectable isotopic differences exist between potential diet constituents (Ramsay and Hobson 1991; Abend and Smith 1997; Hobson *et al.* 1997b). Furthermore, this quantification can occur non-lethally if tissues are accessed by field methods which have been developed to take, with minimal harassment, biopsies from free-ranging cetaceans (Weinrich *et al.* 1991; Lambertsen *et al.* 1994). This non-lethal aspect is essential in experimental designs that use repeated sampling of same individuals to assess diet variability with time (Schell *et al.* 1989a; Abend 1993; Borobia *et al.* 1995; Ames *et al.* 1996; Hobson *et al.* 1996; Hobson and Stirling 1997). Finally, the technique establishes nutritional dependency on specific dietary items as it measures assimilated carbon or nitrogen, and thus addresses problems of selective assimilation (Ramsay and Hobson 1991; Hobson and Stirling 1997).

1.7 Research Objectives

Current biopsy programs that are relatively non-invasive and non-lethal provide a useful source of tissue for SIA (Borobia *et al.* 1995; Todd *et al.* in press). Methods using SIA have not been extensively applied to humpback whales, yet may provide a non-invasive diet assessment alternative to analysis of gut contents. This thesis uses field biopsy samples taken from the

Newfoundland and Labrador feeding substock of the Northwest Atlantic humpback whale population.

The thesis is divided into two sections. In the first section, methods are developed to analyze isotopically humpback skin tissue taken by biopsy. In the second section SIA is applied to a series of biopsy samples obtained through project YoNAH (Years of the North Atlantic Humpback Whale), an extensive international census program conducted over 3 years to determine the distribution and size of the population of Northwest Atlantic humpback whales (Palsbøll *et al.* 1997; Smith *et al.* in review). Data are examined to delineate trophic differences due to sex and geographic/oceanographic site of sample. Trophic changes as a function of inter- and intra-annual change are also documented. Together these results are interpreted in the context of the foraging ecology of the humpback whale and other balaenopterid species.

The following questions are examined in this thesis:

1. Can a biopsy-based SIA technique be used in studies of cetacean foraging ecology? Specifically, can consistent isotopic compositions be determined from the small sample amounts obtained by field biopsy methods? What tissue fraction of a biopsy is most appropriate for use in SIA ?

2. What range of isotopic values typifies the Newfoundland and Labrador feeding substock of the Northwest Atlantic population of humpback whales? Specifically, how do the following factors affect the isotopic ratios $\delta^{13}C$ and $\delta^{15}N$; sex differences; geographic/oceanographic differences; changes within a feeding season; interannual variation?

3. Are isotopic ratios in humpback whales supported by current isotopic knowledge of known prey?

4. What differences exist between the Newfoundland and Labrador feeding substock of the Northwest Atlantic population of humpback whales and other sympatric species of baleen whales?

2. Can biopsy material taken from free-ranging humpback whales be used in isotope studies[1]?

2.1 Introduction

In 1992–1994, an extensive program of sampling of the Northwest Atlantic population of humpback whales provided a number of biopsies associated with identified individuals around the coasts of Newfoundland and Labrador (YoNAH; Palsbøll *et al.* 1997; Smith *et al.* in review). The biopsy dart typically sampled a 0.5 cm diameter core of the entire layer of skin and occasionally blubber, approximately 2-3 cm in depth, depending on angle of dart entry (described in Matthews *et al.* 1988). To preserve the material for genetic analyses, the biopsy material was archived in dimethyl sulfphoxide (DMSO) (Amos and Hoelzel 1991; Palsbøll *et al.* 1997; Smith *et al.* in review).

To assess the potential of the skin fraction of YoNAH biopsy samples for use in an isotopic analysis program, it was necessary to conduct a preliminary isotopic examination of humpback whale tissues. To do this, multiple samples taken from humpback whales killed in fishing gear (Lien *et al.* 1990, 1991) were analysed to determine:

- Isotopic differences between skin and blubber obtainable in a non-lethal biopsy, and muscle, obtainable only through *post mortem* dissection. Muscle is the most frequently tissue used in SIA studies and it was important to establish that similar isotopic measurements could be obtained from alternative tissue sources. It was expected that $\delta^{13}C$ values of blubber would demonstrate highly variable low values, because of its higher lipid content in comparison

[1]A majority of data in this chapter has been published in the paper "Use of biopsy samples of humpback whales (*Megaptera novaeangliae*) skin for stable isotope ($\delta^{13}C$) determination", by S. Todd, P. Ostrom, J. Lien and J. Abrajano (J. Northw. Atl. Fish. Sci. 22, in press).

with skin and muscle fractions, and because lipids are known to be isotopically depleted in ^{13}C (DeNiro and Epstein 1977).

- The effects of two different lipid extraction techniques on the removal of DMSO from tissue samples. DMSO is a common organic preservative used in samples collected for genetic analysis, and data suggest that it may compromise $\delta^{13}C$ and $\delta^{15}N$ values (Hobson *et al.* 1997a). It was therefore necessary to confirm that the effects of DMSO upon isotopic composition could be controlled.

- Procedural reproducibility for automated isotopic analysis of skin samples of the same size as those obtained by biopsy. Analysis by a non-automated modified Dumas method (Macko *et al.* 1987; Shearer and Kohl 1993) requires 5 mg of dry, homogenized tissue, and is labour-intensive (typically 20 samples per day can be prepared for analysis). Recent advances in automation (Mulvaney 1993) make the process more rapid, and in some cases substantially less tissue is required for analysis (~3.5–4 mg for $\delta^{15}N$, ~0.5 mg for $\delta^{13}C$). However, analyses involving extractions use relatively large samples (> 0.5 g), to account for tissue lossed during the extraction process, and to ensure the isotopic homogeneity of the sample (Boutton 1991). Biopsies usually yield ~50 mg (dry weight) of skin, and ~100 mg of blubber. Confirmation was needed that sufficient biopsy sample remained following these procedures for isotopic analysis.

2.2 Methods

All analysis equipment was thoroughly washed in detergent and rinsed in acetone, then baked at 500°C for 1 hr to combust any residual organics prior to use. Sample preparation tools (scalpel, mortar and pestle, etc.) were treated similarly between each sample.

2.2.1 Tissue comparison of $\delta^{13}C$ and $\delta^{15}N$

Muscle, skin and blubber tissue from four humpback whales found dead in fishing gear (2 males, 2 females; Table 2.1) were taken *post mortem* and frozen for subsequent analysis. Samples were thawed and dried at 40°C to a constant mass. Dried samples were ground to a fine powder using mortar and pestle. Isotope analyses were performed by a modified Dumas method (Macko *et al.* 1987; Shearer and Kohl 1993). In this method, ~5 mg of tissue was combusted in a sealed, evacuated quartz tube in the presence of excess copper and copper oxide.

Gases of suitable purity were obtained by cryogenic separation. Isotopic determination of $\delta^{13}C$ was performed using an OPTIMA stable-isotopic ratio mass spectrometer (VG Isotech, operated by L. Windsor, Department of Earth Sciences, Memorial University of Newfoundland).

Additional muscle and skin samples from six individuals (2 males, 4 females; Table 2.1) were prepared as above using a ball and capsule amalgamator (Wig-L-Bug, Crescent Industries) to homogenize materials. Approximately 5 mg of each sample was placed into individual aluminium cups for determination of $\delta^{15}N$. Measurement of $\delta^{15}N$ was performed using an automated system (Mulvaney 1993) with a Carlo Erba Elemental Analyzer interfaced to a PRISM stable-isotopic ratio mass spectrometer (VG Isotech, operated by P. Ostrom, Department of Geological Sciences, Michigan State University). Data were analyzed in a mixed design two-way analysis of variance (ANOVA), with different tissue types treated as a repeated measure, and sex as a randomized variable. Specific comparisons were conducted *post hoc* using Scheffé criteria (Keppel and Zedeck 1989).

Table 2.1: List of humpback whales killed in fishing gear (Lien *et al.* 1990, 1991) used in preliminary isotopic investigations of various tissues (muscle = m, blubber = b, skin = s).

					Tissues analysed	
Sample No.	Site	Date	Gender	Length (m)	$\delta^{13}C$	$\delta^{15}N$
Mn001	Point May, Burin Peninsula	26 July 1991	female	8.25	mbs	-
Mn002	Portugal Cove South, Trepassey Bay	29 July 1991	female	unknown	mbs	-
Mn003	Mobil Bay, Southern Shore	11 July 1990	female	unknown	-	ms
Mn004	Lower Island Cove, Conception Bay	30 June 1990	male	unknown	mbs	ms
Mn005	New Harbour, Trinity Bay	2 June 1991	male	unknown	mbs	-
Mn007	Lower Island Cove, Conception Bay	30 June 1990	female	unknown	-	ms
Mn011	unknown	6 August 1991	male	unknown	-	ms
Mn012	Cape St. Francis	6 August 1991	female	unknown	-	ms
Mn013	Bellevue, Trinity Bay	26 June 1991	female	unknown	-	ms

2.2.2 DMSO removal

A second set of samples from a male and a female of the above group were soaked in a solution of DMSO and brine for 8hr prior to freezing. Half of these samples were analyzed as described above without additional treatment to remove lipids. The remaining half were thawed, dried and ground, and then lipid-extracted by sonication or soxhlet device. Lipids were removed via sonication using an azeotropic mixture of 87% dicholoro-methane and 13% methanol for 3 hr under agitation from a sonic probe. The resulting solution was centrifuged, the supernatant decanted and the tissue washed in further solvent, and dried at room temperature. After additional homogenization the sample was combusted by the modified Dumas method (Macko *et al.* 1987; Shearer and Kohl 1993). After cryogenic separation of sample gases, isotopic analyses of $\delta^{13}C$ were performed using an OPTIMA stable-isotopic ratio mass spectrometer (VG Isotech, operated by L. Windsor, Department of Earth Sciences, Memorial University of Newfoundland).

Lipid removal via soxhlet extraction was performed using the above azeotropic mixture for 7 hr. In this procedure, ~100 mg of dried tissue were placed in a cellulose filter and subject to repeated washings of clean condensate of the azeotropic solvent mixture. Cryogenic separation and isotopic analysis of $\delta^{13}C$ then proceeded as above. Measurement of $\delta^{15}N$ values was not performed. Data were analyzed using a two-way mixed design ANOVA treating tissue type as a repeated measure and presence or absence of DMSO as a randomized variable. Analysis of the interaction, and simple effects within the interaction, were performed manually using methods prescribed by Keppel and Zedeck (1989) for unbalanced designs. Data examining the removal of DMSO were analyzed using a one-way ANOVA, using Scheffé criteria to test differences between specific treatments *post hoc* (Keppel and Zedeck 1989).

2.2.3 Procedural Reproducibility

A section of skin collected from a stranded humpback whale (unknown sex and location) was dried and divided into 4 aliquots of ~50, ~100, ~250, and ~500 mg dry mass. Each aliquot was homogenized using a ball and capsule amalgamator (Wig-L-Bug, Crescent Industries). Lipids were removed from the homogenates in an azeotropic mixture of 87% chloroform and 13% methanol using soxhlet extraction for 7 hr (this solvent is of a similar polarity to that used for extractions described in section 2.2.2). Samples were dried in an evaporatory oven and measured into aluminum caps. Analysis of $\delta^{13}C$ and $\delta^{15}N$ was performed using a Carlo Erba Elemental Analyzer interfaced to a PRISM stable-isotopic ratio mass spectrometer (VG Isotech, operated by K. B. Pilichowski, Michigan State University).

2.3 Results

2.3.1 Stable isotopic comparisons between tissues and sexes

Results of the isotopic analysis for $\delta^{13}C$ are shown in Table 2.2 for 4 individuals. The ANOVA demonstrated no differences between sexes or any interaction between sex and tissue type; that is, differences between tissue types were similar for both males and females. However, significant differences were found between tissue types ($F = 15.27$; df = 2, 6; $p < 0.01$). These differences were primarily between blubber ($\bar{x} \pm SD$ = -23.7 ± 0.2‰) and muscle/skin considered together (Scheffé test, $p < 0.01$), but not between muscle (-19.1 ± 0.7‰) and skin (-19.5 ± 0.5‰; Scheffé test, $p = 0.50$).

Results of the isotopic analysis for $\delta^{15}N$ are shown in Table 2.3 for 6 individuals. No difference was detected between muscle ($\bar{x} \pm SD$ = 13.7 ±1.2‰) and skin tissue (13.3 ±1.6‰; F =

Table 2.2. Stable-carbon isotopic values ($\delta^{13}C$) for skin, muscle and blubber tissues sampled from 2 male and 2 female humpback whales. The $\delta^{13}C$ value for blubber in female 1 was not available.

		$\delta^{13}C$ (‰)		
Sample No.	Sex	Muscle	Blubber	Skin
Mn001	female	-20.0	-	-19.8
Mn002	female	-18.9	-23.5	-19.9
Mn004	male	-18.5	-23.7	-18.9
Mn005	male	-18.9	-23.8	-19.5

Table 2.3. Stable-nitrogen isotopic values ($\delta^{15}N$) for skin and muscle taken from 6 humpback whales.

		$\delta^{15}N$ (‰)	
Sample No.	Sex	Muscle	Skin
Mn003	female	14.1	14.2
Mn004	male	11.9	10.3
Mn007	female	12.7	13.0
Mn011	male	14.0	13.7
Mn012	female	15.1	14.3
Mn013	female	14.2	14.4

3.30; df = 1, 4; $p = 0.14$) in values of $\delta^{15}N$. This lack of difference was consistent for both males and females.

2.3.2 Effects of DMSO on $\delta^{13}C$

Six individuals were tested for the effects of the addition of DMSO on $\delta^{13}C$ values. A significant interaction between DMSO treatment and tissue type ($F = 24.39$; df = 2, 6; $p < 0.01$) indicated that the effects of DMSO were not consistent across tissue types (Table 2.4). Further examination of the simple effects of this design demonstrated that the addition of DMSO significantly reduced values of $\delta^{13}C$ for both muscle ($p < 0.01$) and skin ($p < 0.025$) tissues (Table 2.4). However, DMSO did not significantly change the value of $\delta^{13}C$ in blubber ($p = 0.23$), either because the $\delta^{13}C$ values for DMSO and blubber are similar, or because the carbon isotopic composition of the blubber fraction dominated the isotopic composition of DMSO.

Both soxhlet and sonication lipid-extraction techniques resulted in isotopically higher $\delta^{13}C$ values than those of non-extracted tissues, because of the removal of ^{13}C depleted lipids (Table 2.5; Scheffé test, $p < 0.01$ for either method). Lipid extraction also significantly increased $\delta^{13}C$ values of DMSO treated samples ($\delta^{13}C$ = -18.3‰) when compared to non-lipid extracted DMSO samples ($\delta^{13}C$ = -20.7‰; Scheffé test, $p < 0.01$). Importantly, there was no significant difference between a lipid-extracted DMSO-treated sample ($\delta^{13}C$ = -18.3‰) and a lipid-extracted non-DMSO-treated sample ($\delta^{13}C$ = -18.5‰; Scheffé test, $p = 0.83$). The soxhlet and sonication techniques resulted in slight (0.3‰) but significant differences in terms of final $\delta^{13}C$ value (Scheffé, $p < 0.05$). These differences approach the level of procedural reproducibility (see below), and are likely related to the efficiency of the extraction technique.

Table 2.4. Stable-carbon isotopic values ($\delta^{13}C$) for DMSO (n = 2) and non-DMSO (n = 4) treated tissues, averaged across sex.

	$\delta^{13}C$ (‰), $\bar{X} \pm SD$	
Tissue	Non-DMSO	DMSO
muscle	-18.8 ± 0.3	-21.9 ± 0.2
blubber	-23.7 ± 0.2	-23.4 ± 0.3
skin	-19.4 ± 0.2	-21.4 ± 0.2

Table 2.5. Stable-carbon isotopic values ($\delta^{13}C$) for skin biopsy material treated for removal of DMSO by various methods of lipid-extraction.

Treatment	Extraction technique	$\delta^{13}C$ (‰)
Control	none	-19.8
Control	sonication	-18.4
Control	soxhlet	-18.6
Control with DMSO	none	-20.7
Control with DMSO	sonication	-18.3

2.3.3 The effects of sample amount on reproducibility

Sample amount did not significantly affect values for either $\delta^{13}C$ ($r = 0.38$; $n = 7$; $p = 0.41$) or $\delta^{15}N$ ($r = 0.57$; $n = 7$; $p = 0.18$), expressed as deviations from the sample mean (Figure 2.1). In fact, the automated technique produced highly consistent results for both $\delta^{13}C$ ($\bar{x} \pm SD$: -18.8 ± 0.1‰) and $\delta^{15}N$ ($\bar{x} \pm SD$: 14.2 ± 0.1‰).

2.4 Discussion

2.4.1 Comparisons of stable isotopic values with previous SIA studies

Data from this preliminary examination agree with previous isotopic analyses of cetaceans (Schell *et al.* 1989a; Ostrom *et al.* 1993; Abend and Smith 1995; Borobia *et al.* 1995; 1997), and other marine mammal predators (Ramsay and Hobson 1991; Hobson and Welch 1992; Rau *et al.* 1992; Hobson *et al.* 1996, 1997b). Specifically, the non-lipid-extracted $\delta^{13}C$ value for muscle of ~19‰ found in this study shows is similar to Ostrom *et al.*'s (1993) $\delta^{13}C$ non-lipid-extracted value for humpback whale muscle of -18.7‰ ($n = 1$), and falls within a range typical of many of the baleen whales sampled in that same report.

The lipid-extracted skin $\delta^{13}C$ value of -18.6 ± 0.02‰ ($\bar{x} \pm SD$) found in this study is also similar to previous estimates of lipid-extracted muscle ($\delta^{13}C$ = -17.8 ± 0.4‰, $n = 11$) from humpback whales found stranded or caught in fishing gear around Newfoundland (Ostrom and Lien, unpub. data). Values of muscle $\delta^{15}N$ from this study ($\delta^{15}N$ = 13.7 ± 0.5‰) are similarly in agreement with those previous published ($\delta^{15}N$ = 13.4‰, $n = 1$; Ostrom *et al.* 1993) and unpublished ($\delta^{15}N$ = 13.6 ± 0.9‰, $n = 15$; P. Ostrom and J. Lien unpub. data) for humpback whales in Newfoundland and Labrador waters. Variability in isotopic ratios among whales

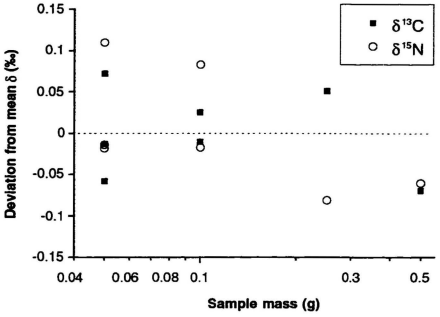


Figure 2.1. Reproducibility of $\delta^{13}C$ and $\delta^{15}N$ values as affected by sample amount, expressed as absolute deviations from their respective sample means. No statistical relationship was seen for either isotopic variable, suggesting signal homogeneity was preserved in smaller sample sizes.

and studies is likely a function of geographic area, time of year, and diet, as demonstrated by Schell *et al.*, (1989a) for bowhead whales.

Results from the reproducibility tests indicated that working with small sample masses similar to those obtained in a dart biopsy did not effect the reproducibility or accuracy of stable isotopic measurements. Sufficient sample is retained post-lipid extraction for analysis purposes, if an automated method is used. While the necessary sample homogeneity could be obtained with respect to within-sample measurements, this data could not assess within individual reproducibility—that is, between samples taken from the same tissue of the same individual.

Most isotopic analyses report precisions of 0.2–0.3‰ for either $\delta^{13}C$ or $\delta^{15}N$ (Boutton 1991; Mulvaney 1993). Data from this study suggest greater precision can be obtained, perhaps ~0.1‰ for either isotopic variable. However, for the purposes of this thesis, a precautionary approach is taken, and measurement resolution is assumed to be 0.2‰.

2.4.2 Stable-isotopic composition and metabolic turnover

Differences in biochemical composition between tissue types can result in differences in isotopic composition within an individual (Gearing 1991). Alternatively, differences in isotopic composition may be a function of differing tissue integration times, if tissues have different turnover rates and diet changes within that period (Tieszen *et al.* 1983; Hobson and Clark 1992a). No data are available for turnover rates of various tissue types in cetaceans, but some studies rank tissue turnover in order of metabolic activity (Jones *et al.* 1981; Fry and Arnold 1982; Tieszen *et al.* 1983; Boutton *et al.* 1988; Hobson and Clark 1992a). Tissue-specific metabolic rates are available for several vertebrates (Altman and Dittmer 1968), and also specifically for several metabolites (Waterlow *et al.* 1978; Hetenyi *et al.* 1983). When plotted, such data indicate liver has high metabolic activity, blubber has low metabolic activity, and muscle and

skin have intermediate rates (Figure 2.2; Altman and Dittmer 1968). Data on protein metabolism in rats (*Rattus* sp.) confirm that turnover periods are greater in liver (half-life ~2 days) than in muscle (~14 days; Waterlow *et al.* 1978). Fewer data are available on skin, although Waterlow *et al.* (1978) suggest that skin turnover is approximately half to two-thirds that of liver tissue.

Experiments in protein metabolism indicate that skin has a faster rate of protein synthesis than muscle in both cattle (*Bos primigenius*) and sheep (*Ovis ammon*; Lobley *et al.* 1980, 1992; Attaix *et al.* 1988). Thus, in marine mammals, skin tissue likely has a shorter turnover time than muscle and reflects shorter-term dietary information (Hobson *et al.* 1996). Given the importance of blubber as a food reserve in non-feeding months (Lockyer 1981), in non-fasting animals blubber likely has a longer turnover period than muscle and reflects longer term dietary information (Hobson and Stirling 1997). Thus, in order of increasing turnover rate, it can be hypothesized that:

$$\text{blubber} < \text{muscle} < \text{skin}$$

Although such statements concerning turnover rate are often made (for example, Abend and Smith 1995), data for cetaceans are lacking. Many studies use Tiezen *et al.*'s (1983) finding of a half-life of 27 days for muscle tissue taken from gerbils (*Meriones unguienlatus*). The applicability of such a figure to marine mammals remains questionable (Hobson *et al.* 1996). Hobson and Clark (1992a) quote a briefer period of 12 days for quail (*Coturnix japonica*). However, Tiezen *et al.*'s data serve as a first approximation for mammals. Overall, data support the above ranking of tissues, at least for non-fasting animals. For fasting animals, this relationship will likely change as fat reserves are mobilized and decreased protein utilization occurs (Lockyer 1987; Castellini and Rea 1992; Markussen *et al.* 1992b). Thus during fasting periods, blubber may have a briefer turnover rate than some muscle tissue.

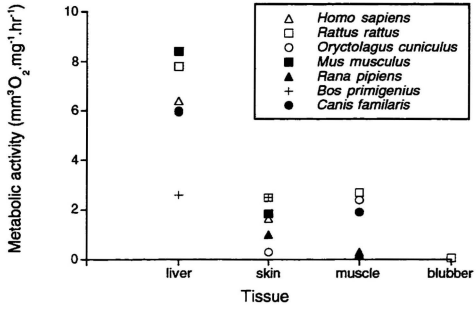


Figure 2.2. Non-fasting metabolic activities of various tissues for seven vertebrate species (from Altman and Dittmer 1968).

In this thesis, which examines non-fasting humpbacks, it is assumed that the turnover period for muscle tissue is ~30 days. Since evidence suggests that skin tissue has a briefer turnover period than muscle, this study further assumes that the turnover period for skin is < 30 days. This latter assumption is supported by data taken from captive manatees; longitudinal time-series of manatee skin isotopic levels were found to lag behind dietary isotopic levels by a period of 30–60 days (Ames *et al.* 1996), suggesting a half-life for skin of ~30 days or less.

While slight isotopic differences between muscle and skin were found, these were statistically indistinguishable for individuals measured in this study. For pilot whales, Abend and Smith (1995, 1997) demonstrated similar, yet statistically significant differences between skin and muscle $\delta^{15}N$ (0.2–0.5‰) values: however, non-lipid-extracted $\delta^{13}C$ differences between muscle and skin (0.8‰) were higher than found in this study. In both cases the authors note that these differences may be due to differential feeding within the turnover period of the tissues examined.

Two individuals in this study showed large differences between muscle and skin $\delta^{15}N$ values (> 0.8‰, individuals Mn004 and Mn012; Table 2.2). Given that both muscle and skin are low in lipids, such a difference may reflect different diets taken within the isotopic feeding history of the animal (Abend and Smith 1997). Thus, the data suggest that these specific animals would have recently fed on a lower trophic level dietary source, assuming $\delta^{15}N$ can be used as a proxy for trophic level. Alternatively, since both were found dead in fishing gear, the increased $\delta^{15}N$ value might indicate protein mobilization within muscle tissues during a forced fast caused by net-entrapment (Gearing 1991; Hobson *et al.* 1993).

2.4.3 Removal of lipids

Lipids tend to have low $\delta^{13}C$ values; thus the observation that blubber has lower $\delta^{13}C$ values (~4.5‰) than either muscle or skin is expected (DeNiro and Epstein 1977). In addition,

blubber values from the present study ($\bar{x} \pm SD$: -23.7 ± 0.2‰, n = 3) are close to estimates by Borobia *et al.* (1995). Because the lipid fraction of a sample tends to be isotopically low, it is expected that a non-lipid-extracted sample would yield a lower $\delta^{13}C$ than a lipid-extracted sample, as indicated in this study.

Weights of lipids extracted from skin samples indicate that the lipid fraction accounts for only 10–15 % of the sample by mass. Thus, the non-lipid-extracted $\delta^{13}C$ value can be mathematically predicted since the overall $\delta^{13}C$ value for the whole tissue will be equal to the isotopic values of individual biochemical fractions weighted by their abundances. This computation is based on the formula:

EQUATION 3:

$$\delta^{13}C_{tissue} = \Sigma(p_i \cdot \delta^{13}C_i)$$

where p_i is the proportional content of constituent *i* (Alexander *et al.* 1996). The protein fraction can be represented by the lipid-extracted value for skin obtained in this study ($\delta^{13}C$ = -18.5‰). The $\delta^{13}C$ value of the lipid fraction can be estimated at ~-26‰ (a mid-range value taken from Borobia *et al.* 1995). If one further assumes a protein:lipid ratio of 85:15 for skin (Chapter 4), a predicted non-lipid extracted value of -19.6‰ can be calculated. This value agrees closely with the measured value of -19.8‰. Such calculations can be used to obtain lipid-corrected data (Alexander *et al.* 1996), in the absence of other organic influences (such as an organic preservative) in the sample.

2.4.4 Removal of DMSO

Hobson *et al.* (1997b) conclude that the presence of DMSO in a sample alters $\delta^{13}C$ and $\delta^{15}N$ values, and thus the authors recommend caution in examining DMSO-treated samples. This finding was demonstrated by soaking samples for 8 hr in a combined solution of DMSO, EDTA and NaCl, and then rinsing in distilled water prior to isotopic measurement. However, a close examination of Hobson *et al.*'s methodology suggests that their conclusions may not be warranted. In fact, it cannot be conclusively shown that a DMSO-treated sample will have an inconsistent isotopic composition because: a) Hobson *et al.* do not show if it is the presence of EDTA, or DMSO, or both, that caused the isotopic alteration (although they hint that EDTA, rather than the DMSO, that was responsible for the isotopic change, and that the latter—as a solvent—increased the permeability of cells to EDTA); b) they cannot demonstrate that rinsing alone removed any residual component of either contaminating preservative (thus they could be measuring the isotopic composition of a tissue sample in the presence of the preservative, rather than a tissue sample chemically-altered by the preservative); and c) they present no data concerning the effects of soak-time in the preservative solution.

This study confirms that the addition of DMSO dramatically changes the $\delta^{13}C$ value for muscle and skin. However, lipid extraction (as opposed to simple rinsing in distilled water as done by Hobson *et al.* above) appears to remove the effects of DMSO (in this thesis the effects of EDTA are irrelevant as it was not used as an additive). Sonication and soxhlet extraction are two alternatives that removed DMSO; either method achieved similar isotopic results. The reduced handling time associated with soxhlet extraction is appealing, both in terms of labour costs and safety. Use of micro-soxhlet devices reduced the loss of sample material and is therefore preferred in the preparation of small (< 0.5 g) samples. In addition to its ability to remove DMSO, lipid extraction has the advantage of removing isotopically unique lipids that can obscure isotopic information retained in the tissues (Ostrom *et al.* 1993).

However, while this thesis assumes that the isotopic effects of DMSO can be neutralized, it should be noted that the chemical interaction beween DMSO and a sample is still poorly understood with respect to isotopic composition. Given the frequent use of DMSO as an organic preservative, this is certainly an area that warrants further investigation.

2.5 Conclusions

The above results indicate that skin biopsies are an appropriate tissue source for SIA. Moreover, it should be possible to analyze biopsies archived in DMSO, using extraction techniques that will also remove noise created by lipid fractions with unique $\delta^{13}C$ values. SIA provides a non-lethal alternative to examination of stomach contents to determine diet. SIA evaluations, based on analyses of assimilated tissues, reflect dietary information integrated over a longer period than the short-term sampling of recently digested food items examined in stomach content analysis. With the added possibility of resampling individually identified individuals within or between seasons, SIA can be incorporated into longitudinal studies of foraging behaviour.

3. Methods - Collection and analysis of samples biopsied from free-ranging balaenopterids

3.1 Location, effort, and photo-identification

This study uses samples and data collected in part by YoNAH, a two-year international project estimating the size of the North Atlantic population of humpback whales, conducted in 1992 and 1993 (Palsboll *et al.* 1997; Smith *et al.* in review). With respect to this study, collection effort for the YoNAH programme was in the inshore (< 20 km) coastal waters of Newfoundland and southern Labrador (below 52° N). YoNAH protocol required the collection of three types of data: a) location and time of sightings of humpback whales and other cetaceans; b) photo-identification of all humpbacks; and c) biopsy sampling of identified humpbacks (Smith *et al.* in review). Thus, the YoNAH database compiled an extensive collection of biopsy samples from known individuals of the Newfoundland and Labrador feeding substock, taken throughout the feeding season.

Primary areas surveyed included (but were not limited to) the Northeast coast of Newfoundland (Bonavista, Trinity, White, and Notre Dame Bays), the West coast of Newfoundland (Rocky Harbour, Newfoundland, north to Henley Harbour, Labrador), the East coast and Southern Shore (Conception, Witless, Trepassey and St. Mary's Bays), and the South coast of Newfoundland (Placentia and Fortune Bays). These areas were arbitrarily labeled Geographic Zones (GZ) 1–5 (Figure 3.1).

Research teams included a skipper and photographer, and used small vessels of similar range and operational capacity (generally up to Beaufort Scale 4). Effort expended per GZ is given in Table 3.1. Unequal effort between areas within a year reflects abundance and distribution of humpback whales, because location of the main densities varied from year to

Figure 3.1. Map of the study area, showing designation of geographic zones (GZs), giving main concentrations of biopsies for YoNAH and non-YoNAH protocols, 1992-1995 (hatched zones).

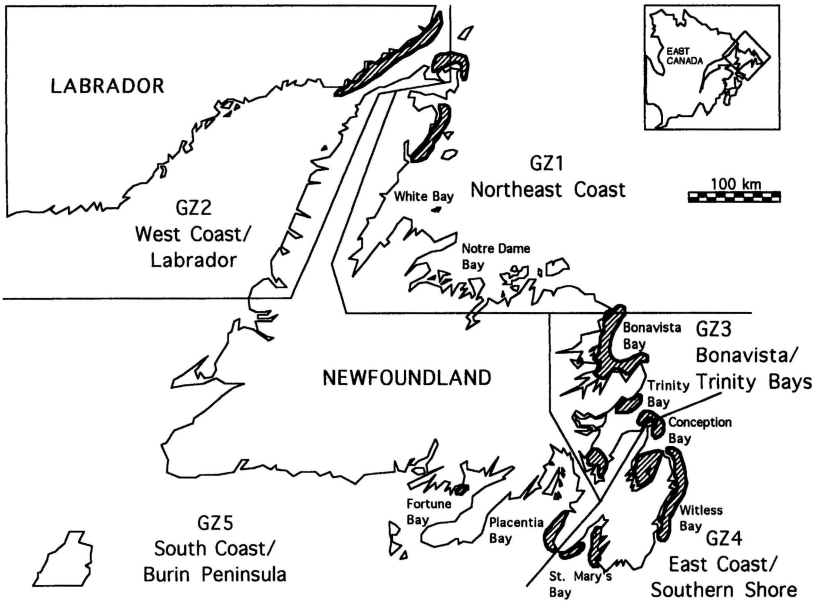

LABRADOR
EAST CANADA
GZ1
Northeast Coast
100 km
GZ2
West Coast/
Labrador
White Bay
Notre Dame
Bay
Bonavista
Bay
GZ3
Bonavista/
Trinity Bays
NEWFOUNDLAND
Trinity
Bay
Conception
Bay
Fortune
Bay
Placentia
Bay
Witless
Bay
GZ4
East Coast/
Southern Shore
St. Mary's
Bay
GZ5
South Coast/
Burin Peninsula

Table 3.1. Total boat effort of 1992-1994 census operations, measured in boat-hours for each GZ. NA indicates census operations during which effort was not logged. Thus, in some cases (indicated by *), totals indicate a minimal estimate of effort due to unlogged effort.

Census effort (boat hrs)

	1992				1993				1994				
GZ	June	July	August	Sept.	June	July	August	Sept.	June	July	August	Sept.	Totals
1	-	NA	-	-	-	22	43	-	-	-	-	41	106*
2	-	-	NA	NA	NA	158	170	-	-	-	-	-	328*
3	6	-	-	-	-	-	43	-	-	103	243	23	418
4	5	101	2	-	35	66	34	-	21	109	10	-	383
5	4	19	15	-	-	66	-	-	74	49	-	-	197
Month totals	15	120*	17*	NA	35*	312	290	-	95	261	253	64	
Year totals		152*				637*				673			1432*

year, and from month to month. Differences in effort between years also reflect whale abundance and logistical considerations.

Weather permitting, research crews would perform a transect each day, searching for groups of animals. Transects were typically of a zig-zag configuration (5-km sections followed by alternating left and right 90° turns). Each cruise logged times and types of activity (search/travel/working), locations and directions of course changes using a differential Global Positioning System (GPS), speed (using GPS), weather conditions and visibility (using Beaufort scale), and whale encounters (specifying minimum/maximum number in group). An effort was made to maximize the number of whales observed but minimize the number of resightings. This was an important principle as one of YoNAH's mandates was to obtain an accurate population estimate; thus, as many different individuals as possible were sampled, maximizing the efficiency of the capture-recapture method used in population estimation (Hammond 1986).

All humpbacks encountered were photographed for the purposes of identification. The pigmentation pattern on the underside of a humpback whale's flukes (exposed during a terminal dive sequence) is permanent and unique to the individual, and can thus be used to identify that animal (Katona *et al.* 1979; Katona and Whitehead 1981; Carlson and Mayo 1990; Hammond *et al.* 1990; Lien and Katona 1990; Mizroch *et al.* 1990). Photographs were taken with a 35 mm SLR camera fitted with a motor-wind and 70–210 mm zoom lens, using 400 ASA black and white film. In addition to fluke ventral pigmentation, researchers also attempted to photograph left and right views of the dorsal fin, which were also used in identification (Katona and Whitehead 1981; Lien and Katona 1990).

3.2 Biopsy collection

Biopsy sampling of free-ranging individuals is now an established technique in cetacean research (Winn *et al.* 1973; Matthews *et al.* 1988; Palsbøll *et al.* 1991; Lambertsen *et al.* 1994).

Developed for collection of material for genetic analysis (Winn *et al.* 1973; Lambertsen *et al.* 1988; Palsbøll *et al.* 1992), it is now recognized that tissues from biopsies are useful for other analyses—including fatty-acid analysis (Borobia *et al.* 1995) and stable-isotopic analysis (Borobia *et al.* 1995; Todd *et al.* in press). The technique is minimally invasive and is thought not to produce any long-term behavioural effects (Weinrich *et al.* 1991). In this study, biopsy collection was not attempted until photo-identification of most individuals within a sighting were obtained. Animals were then approached from behind, and a biopsy dart fired using a 150-lb-draw crossbow at a distance of < 30 m (Figure 3.2; Matthews *et al.* 1988). The dart typically sampled skin (and sometimes blubber) from the dorsal side of the animal, posterior to the dorsal fin.

Tissue collected from the biopsy dart was transferred using forceps to a sterile vial containing a mixture of brine and dimethyl sulfphoxide (DMSO), used to preserve the integrity of the tissue (Amos and Hoelzel 1991). Sterilization of darts and forceps between biopsies was by consecutive, separate rinsings in hydrogen peroxide and distilled water. All biopsy samples were kept on ice wherever possible until they could be transferred to a freezer (-30 °C), and divided for the purposes of sexing and SIA. Sex was determined by P. Palsbøll (Department of Population Biology, University of Copenhagen, Denmark) using a polymerase chain reaction technique (Palsbøll *et al.* 1992).

3.3 Additional non-YoNAH sampling procedures

The biopsy database was augmented with samples collected outside of YoNAH procedures and years. In chronological order of sampling, these included:

- Samples taken opportunistically from strandings in 1988 (n = 1), 1990 (n = 8) and 1991 (n = 6). These samples were taken *post mortem*, from an area close to the dorsal fin, and had been analyzed prior to this study (J. Lien and P. Ostrom unpub. data).

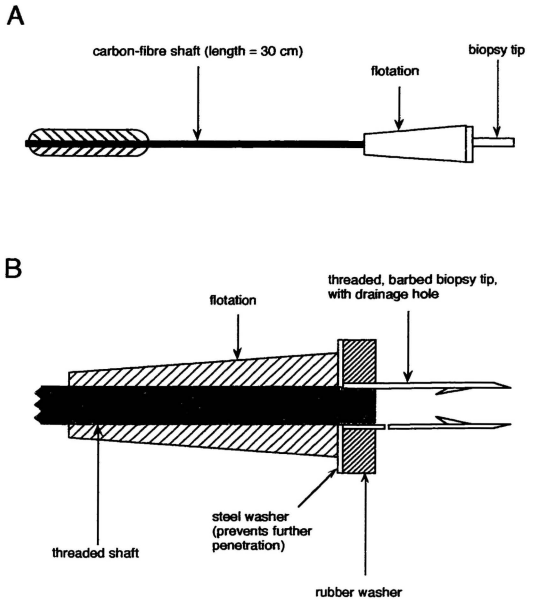


Figure 3.2. Diagram of the biopsy dart used to obtain samples from free-ranging cetaceans (A) with actual-size cross-section detail of the biopsy tip (B). The dart was deployed using a 150-lb-draw crossbow (Matthews *et al.* 1988).

- Samples obtained during non-YoNAH cruises in 1992 and in 1994. Protocols followed those used in the YoNAH project, with some exceptions. All biopsies taken in 1994 were divided into DMSO and non-DMSO (brine only) fractions, the former to be used for genetic analysis, and the latter for isotope analyses. When possible, biopsy attempts were made on species other than humpback whales, including finback and minke whales. Finback whales were comparatively easy to biopsy. Minke whales, with their somewhat erratic and brief surfacing pattern, could not be biopsied within the time allotted to each whale (Section 3.4), although sloughed skin from the tail of a net-entrapped minke whale was obtained and analyzed.
- A sample of sloughed skin collected from an ice-entrapped humpback whale, 21st April 1995, in Brownsdale, Trinity Bay (GZ3).

3.4 Ethical considerations

Photo-identification procedures can be conducted with minimal disturbance to the animal, and some data suggest that humpback whales seldom show a behavioural reaction to a biopsy dart. For example, Weinrich *et al.* (1991) demonstrated that only 3% of their biopsy attempts on humpback whales within the Gulf of Maine feeding substock resulted in an adverse reaction (repeated tail-flicks, surges, etc.). In contrast, humpback whales in the Newfoundland feeding substock usually react to biopsy attempts with behavioural responses such as rapid dives, tail-flicks, and breaching (J. Lien pers. comm.). Thus, to minimize harassment, a protocol was adopted that precluded collection of data beyond 10 terminal dives or 45 min of effort on any one animal (whichever occurred first). If an animal displayed signs of harassment before this period ended, collection would only proceed at the discretion of the research team leader. In addition, an attempt was made to sample an individual only once, thus minimizing the disturbance per animal.

All the above procedures were approved on a yearly basis by the Animal Care and Ethics Committee of Memorial University of Newfoundland and the YoNAH Executive Committee (chaired by S. Katona, AlliedWhale, College of the Atlantic, Bar Harbor, Maine). All YoNAH procedures were approved by the U.S. National Marine Fisheries Service (permit # 787). Transfer of biopsy material to analysis laboratories required the approval of the above techniques by CITES (permit # CA-FO-SJ-0001-95), and U.S. Fish and Wildlife Management (permit # US-798944).

3.5 Sample selection and preparation, and determination of stable-isotopic composition

Collectively, from 1992-1994, YoNAH and non-YoNAH projects involved over 1400 hours of boat effort (Table 3.1). Biopsies were taken within an area defined by 46° 36′ 23.4″ N to 52° 04′ 03″ N , and 52° 37′ 15.6″ W to 57° 19′ 25.8″ W (Figure 3.1). The YoNAH project collected 272 humpback biopsy samples for the Newfoundland/Labrador region (Table 3.2), with an additional 20 samples collected in 1992 outside of YoNAH protocol. In 1994, 64 humpback and 5 finback whales were sampled; sloughed skin from the tail of a net-entrapped minke whale was also taken. In 1995, an ice-entrapped humpback whale was additionally sampled.

A random subsample of 133 samples was selected, stratified to equalize representation between levels of specified independent variables—specifically, sex, area (GZ), season and year (Section 1.7; Table 3.3). Samples were thawed, and any blubber remaining from the initial biopsy removed. Samples were then dried to constant mass in a ventilated oven (40 °C) and powdered using acombination of a ball and capsule amalgamator (Wig-L-Bug, Crescent Industries) and a heavy duty mortar and pestle. Homogenized samples were stored in air-tight vials in a freezer (-30 °C).

Table 3.2. List of humpback whale biopsies taken per year, month and GZ. Figures given are counts for males/females by both YoNAH and non-YoNAH protocols. Multiple samples were obtained for 8 of the individuals in GZ3 in 1992. One individual sampled during an ice entrapment in GZ3 in 1995 is not included here.

Biopsy count (male/female)

	1992				1993				1994				
GZ	June	July	August	Sept.	June	July	August	Sept.	June	July	August	Sept.	Totals
1	-	4/0	4/4	-	-	-	7/8	-	-	-	-	-	15/12
2	-	-	2/3	18/13	0/2	4/8	7/9	-	-	-	-	-	31/35
3	14/10	19/15	1/0	-	-	-	15/14	-	-	4/0	1/8	-	54/47
4	2/2	10/10	2/5	-	10/5	10/22	5/9	-	-	24/14	3/2	-	66/69
5	-	1/0	2/0	-	-	6/10	-	-	4/3	1/0	-	-	14/13
Month totals	16/12	34/25	11/12	18/13	10/7	20/40	34/40	-	4/3	29/14	4/10	-	
Year totals		79/62				64/87				37/27			180/176

Table 3.3. List of humpback biopsy subsamples used in isotopic analyses, by sex (counts are for males/females), month, year and geographic zone (GZ). Numbers indicate n per treatment condition, selected by random stratified sampling. In 1994, two other balaenopterids were sampled: Bp = finback whale; Ba = minke whale. Twenty humpback whale samples taken in GZ3 in 1992 (11 males/9 females) represent resampling of same-individuals. One humpback whale sampled during an ice entrapment in GZ3 in 1995 is not included here.

Biopsy subsamples (male/female)

	1992				1993				1994				
GZ	June	July	August	Sept.	June	July	August	Sept.	June	July	August	Sept.	Totals
1	-	2/0	1/1	-	-	-	2/2	-	-	-	-	-	5/3
2	-	-	1/1	5/5	0/2	2/2	2/2	-	-	-	-	-	10/12
3	14/10	2/2	1/0	-	-	-	2/2	-	-	4/0	1/6 (+1Ba)	-	24/20 +1Ba
4	2/2	2/2	1/1	-	4/4	2/2	-	-	- (+1Bp)	2/3 (+4Bp)	3/2	-	16/16 + 5Bp
5	-	1/0	4/0	-	-	4/4	-	-	4/3	-	-	-	13/7
Month totals	16/12	7/4	8/3	5/5	4/6	6/6	8/8	-	4/3 (+ 1Bp)	6/3 (+ 4Bp)	4/8 (+ 1Ba)		
Year totals	36/24				18/20				14/14 (+5Bp, 1Ba)				68/58 (+5Bp, 1Ba)

Samples were lipid-extracted using mini-soxhlet apparatus to remove both ^{13}C depleted lipids and the DMSO (preserving agent; Chapter 2). To accomplish this, tissues were weighed (≤ 0.5 g) in cellulose thimbles and placed in soxhlet apparatus using forceps to prevent contamination. Samples were then extracted by repeated rinsing in an azeotropic mixture of 87% chloroform and 13% methanol for 7 hr.

Following extraction of lipids, thimbles were dried in an evaporatory oven. Once dry, samples were removed and reweighed to ensure adequate yield for further analysis. Lipid abundance was determined for approximately half the sample (randomly selected). To accomplish this, the lipids were isolated from the soxhlet solvent by rotary evaporation and weighed.

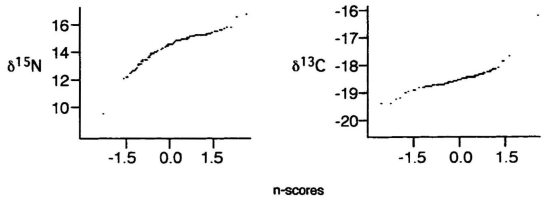
Samples were weighed into aluminum caps (for $\delta^{13}C$ measurements, ~5 mg; for $\delta^{15}N$ measurements, ~3.5 mg). Analysis of $\delta^{13}C$ and $\delta^{15}N$ was performed using a Carlo Erba Elemental Analyzer interfaced to a PRISM stable isotope ratio mass spectrometer (Appendix B; VG Istotech, operated by K. A. Pilichowski and P. Ostrom, Michigan State University). Stable-carbon and nitrogen isotopic ratios were obtained in separate runs. Measurement of samples was interspersed (every 10 samples) by a homogenous preparation of squid (*Ommastrephes bartrami*) of known isotopic composition, to ensure consistent calibration of results. Results from these calibration measurements suggested precision within ±0.1‰ for both $\delta^{13}C$ and $\delta^{15}N$ (but see Section 2.4.1 for a discussion of precision).

3.6 General data analysis and statistical procedures

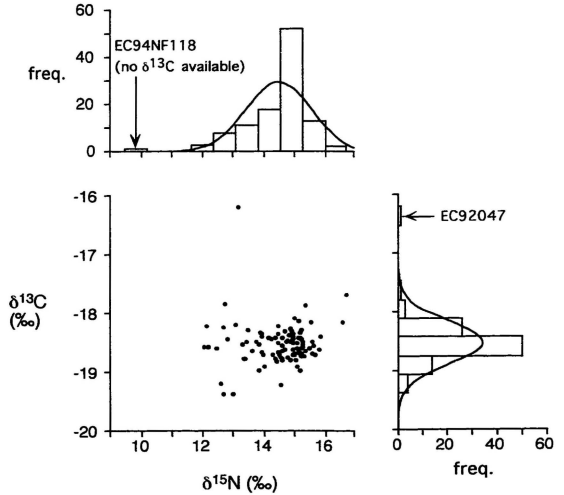
Hobson and Schwarcz (1986) suggest that for animals fed a homogenous diet, isotopic variables are normally distributed. Normal probability plots of data collected in this study indicated that both isotope variables displayed distributions that were close to normal (Figure 3.3), with the exception of two outliers, EC92047 and EC94NF118 (Grubbs test for outliers, $p <$

Figure 3.3. Frequency distributions for $\delta^{15}N$ and $\delta^{13}C$ values for humpback whales, showing a) normal probability plots (where a normal distribution is represented by a straight line), and b) a plot combining frequency histograms for each variable and paired values where both isotopic values are known for an individual whale. Values of $\delta^{15}N$ are positively skewed. Values of $\delta^{13}C$ are negatively skewed. Skewness of distributions is due in part to outliers as labelled. Normal fits to frequency histogram data are displayed on each distribution as a solid curve.

a)


δ15N
16
14
12
10
-1.5
0.0
1.5
δ13C
-16
-17
-18
-19
-20
-1.5
0.0
1.5
n-scores

b)


freq.
60
40
20
0
EC94NF118
(no δ13C available)
δ13C
(‰)
-16
-17
-18
-19
-20
10
12
14
16
δ15N (‰)
EC92047
0
20
40
60
freq.

0.01 for either case; Sokal and Rohlf 1981). These two outliers were removed from subsequent analysis to improve the normality of the isotopic distributions, and are considered as special cases below.

Descriptive statistics of both isotope variables included mean and standard deviation. Variability between $\delta^{13}C$ and $\delta^{15}N$ values was compared by calculation of the coefficient of variation and its standard error for each isotope ($CV \pm SE_{CV}$; Sokal and Rohlf 1981). Measurements of both isotope variables for each whale were not always available. This was usually because the size of the biopsy sample was inadequate to conduct both isotope measurements. Separate 3-way analyses of variance (ANOVA) using $\delta^{13}C$ and $\delta^{15}N$ as dependent variables, and sex, geographic area and year as independent variables, indicated no interactions between variables. Thus, each independent variable was treated separately.

Analysis of each treatment variable was conducted in two parts. In the first part, both isotope variables were considered together as a Combined Isotopic Grouping (CIG; for similar types of analysis, see Hobson 1993; Hobson *et al.* 1994; 1997). Thus, multivariate analysis of variance (MANOVA) was used to determine if treatment levels could be separated by knowledge of both $\delta^{13}C$ and $\delta^{15}N$ (Sokal and Rohlf 1981). In the second part, effects of either isotope variable were considered independently. The type of statistical test used in this secondary analysis depended on examination of the residuals created by the initial MANOVA. In the case of normally-distributed residuals, testing was by analysis of variance (ANOVA), or regression as appropriate, using Scheffé tests *post hoc* to determine differences between treatment means (Sokal and Rohlf 1981). Typically, analysis of the $\delta^{13}C$ variable was parametric because of normally-distributed residuals (Lillifors Test for Normality, $T_2 = 0.92$, $p = 0.37$; Sokal and Rohlf 1981).

If residuals from the intial MANOVA were not normally distributed, testing was by nonparametric analysis; either Mann-Whitney's U (for 2 levels of treatment), Kruskal-Wallis's H (for >2 levels of treatment), or Spearman's ρ (for correlational analyses), as

appropriate (Sokal and Rohlf 1981). Nonparametric tests were commonly used in analyzing the $\delta^{15}N$ variable (Lillifors Test for Normality, $T_2 = 1.41$, $p = 0.03$; Sokal and Rohlf 1981).

For some treatment variables, a third dependent variable, percentage lipid, was considered. As proportional data are not normally distributed, analysis of lipid content was by nonparametric *z*-tests.

The null hypothesis rejection criterion for all analyses was set at $\alpha = 0.05$, except in cases of nonparametric *post hoc* pairwise comparisons. In such cases, a conservative rejection criteria ($\alpha = 0.01$) was adopted in lieu of a more vigorous testing procedure. Three computer statistical packages were used for analysis; *SPSS* for Macintosh v.6.1 (SPSS Inc., 444 Michigan Avenue, Chicago, IL, 60611), *Statview* v.4.51 (Abacus Concepts, 1984 Bonita Avenue, Berkley, CA, 94704), and *DataDesk* v.5.0 (Data Description Inc., Po Box 4555, Ithaca, NY, 14852).

3.7 Specific treatment considerations

The assignment of GZs to data was initially performed to correspond to convenient geographic boundaries used during transects (sampling was typically on a per-bay basis). However, because it was recognized that this process might result in arbitrary geographic division, a comparison was performed that combined GZs on an oceanographic basis; specifically, GZs 1 and 3 (North/East coast), and GZs 4 and 5 (Southern Shore/South Coast). In addition, a secondary analysis was performed using a general linear model (Pedhazur 1997) to investigate the effects of latitude and longitude of sightings (random, continuous independent variables) on CIG. Separate multiple regressions examined individual effects of latitude and longitude of sighting upon either isotope variable (Pedhazur 1997).

Examination of intra-annual variability was initially conducted for the years 1992, 1993 and 1994 separately. The treatment variable was expressed in two separate forms: by month (four

levels of treatment, June-September); and by Day of Year (DoY; representing date within a year). Analyses were conducted as noted above using either expression of time within the year.

The intra-annual treatment variable (in either form) is, in fact, a proxy for a progression of biological events within a seasonal cycle. The annual onset of this cycle is variable, depending on a number of oceanographic and biological factors. Thus, to examine effects pooled across the three years, it was recognized that data may not be synchronized per year. Two approaches were used to biologically justify the pooling of the three years. First, expression of the intra-annual variable as monthly data (a coarse resolution time scale) obscured some of the differences in biological phase between years. Second, synchronization was attempted using annual capelin distribution data to monitor between-year differences in timing. Two aspects of capelin data for beaches within YoNAH-monitored areas were used; both time of first spawning, and time of first peak abundance (Table 3.4; B. Nakashima unpub. data; Nakashima and Winters 1995). Thus, the DoY variable was adjusted to a date relative to capelin timing before the three years of data were pooled.

To complete the examination of intra-annual variation in isotopic signature, eight individuals resampled at intervals of 1–10 days in June, 1992, were analyzed. Biopsies were taken during the whales' residency in Witless Bay (47.78°N, 53.85°W; GZ = 4). To examine if isotopic composition changed within the sampling period, a repeated-measures *t*-test was used to test the null hypothesis that isotopic composition ($\delta^{13}C$, $\delta^{15}N$) did not change during the resampling interval. However, these tests were confounded by varying resampling intervals for each individual.

The data from the eight resampled individuals were also used to obtain an approximation of sampling error within tissues; specifically, to estimate signal homogeneity within skin tissue. This was done by correlating absolute changed in isotope signal ($|\Delta\delta^{13}C|$, $|\Delta\delta^{15}N|$) as a function of resampling interval (ΔDays) for each individual. It was hypothesized that if sampling error was large enough to mask differences due to changes in isotopic composition of

Table 3.4. Two estimates of timing differences in the annual biological cycle (referenced to 1992; $\bar{x} \pm SD$) taken from capelin distribution data. Dates of two biological events—time of first spawning, and time of first peak abundance—were compared for years 1992–1994 to assess phase differences in the biological cycle (measured in days; B. Nakashima, unpub. data; Nakashima and Winters 1995). Estimates of timing differences by first spawning include range (in brackets).

Measure	Timing difference referenced to 1992 (days), $\bar{x} \pm SD$	
	1993	1994
first spawning (n = 6)	-3.2 ± 8.2 (-15 – +10)	+2.8 ± 16.8 (-19 – +19)
first peak abundance (n = 7)	-2.3 ± 5.7	2.0 ± 1.0

the diet, then isotopic values of the consumer would be distributed randomly as a function of resampling interval, and thus no correlation would be evident between either $|\delta^{13}C|$ or $|\Delta\delta^{15}N|$ and ΔDays. However, if diet isotopic effects were greater than sampling error caused by signal heterogeneity within a tissue, significant correlations between either $|\Delta\delta^{13}C|$ or $|\Delta\delta^{15}N|$ and ΔDays would be seen.

Initial analysis of interannual variation in isotopic signature used data collected between 1992-1994. Individuals sampled in 1988-91 (Section 3.3) were subsequently added to the annual dataset to examine long-term trends (P. Ostrom and J. Lien unpub. data). *Post hoc* comparisons were performed pairwise using Mann Whitney's *U*, protected by a conservative rejection criteria ($\alpha = 0.01$). Regressions using Pearson's correlation coefficient ($\delta^{13}C$) and Spearman's Rho ($\delta^{15}N$) were used to confirm long-term annual trends in isotopic ratios.

In 1994, two other balaenopterid species were sampled—5 finbacks and 1 minke whale. These data were used to compare differences in isotopic signature between species. Differences in sample size limited comparisons to non-parametric methods (Mann Whitney's *U*). One-sample z-tests were used to determine if the sole minke whale $\delta^{15}N$ value could be distinguished from the $\delta^{15}N$ values available for either finback or humpback whales. *Z*-tests for proportions were used to compare lipid content data for each species.

3.8 Outliers

Three biopsies taken from humpback whales had isotope values sufficiently different from the population, or were collected under sufficiently different conditions, to warrant special consideration:

1. Sample EC92047 ($\delta^{13}C$ = -16.2‰) was defined as an outlier and excluded from further analysis. Observation of samples taken at similar times (e.g. EC92189; $\delta^{13}C$ = -18.6‰) or in

similar geographic areas (e.g. EC92008; $\delta^{13}C$ = 18.7‰) further supports the removal of this datum. However, both neighbouring examples are males, whereas EC92047 is a female (although other females do not have $\delta^{13}C$ values this high). Sample EC92047 also had the lowest lipid concentration by mass (6.7%) measured for any humpback in this study (but not significantly different from the mean lipid level; $z = -0.83$, $p = 0.08$).

2. Sample EC94NF118, for which only one isotope measurement was available ($\delta^{15}N$ = 9.5‰), was also designated as an outlier. Other animals sampled within the same cruise, time and geographic area, and of the same sex, do not show such depressed levels of $\delta^{15}N$ (e.g. EC94NF113; $\delta^{15}N$ = 15.1‰).

Removal of sample EC92047, EC94NF118 or both from the data set had no effect on subsequent data analysis. However, the removals substantially improved the normality of either isotope distribution (Figure 3.3), thus providing further statistical justification for parametric testing.

3. A third sample, EC94NF110, taken on DoY = 105 (1995; GZ3), was excluded from all analyses because of the circumstances of its collection. The animal was in poor condition and ice-entrapped (and therefore unfed) for at least 2 days. The animal was likely under great physiological stress. Sample collection was from sloughed tissue, thus representing the outermost layer of skin only. This animal's isotopic values ($\delta^{15}N$ = 11.2‰, $\delta^{13}C$ = -18.1‰) were significantly lower than the 1992-1994 population with respect to $\delta^{15}N$ but not $\delta^{13}C$ ($p < 0.01$ and 0.32 respectively; z-test).

3.9 Prey isotopic composition

Isotopic data for prey sampled within the Northwest Atlantic from published (Dickson 1987; Hobson and Montevecchi 1991; Ostrom *et al.* 1993; Hobson *et al.* 1996) and unpublished sources (S. Todd unpub. data; J. Lawson and V. Lesage unpub. data) are given in Table 3.5. A majority of this data comes from species found in Newfoundland waters, although data from Georges Bank (Massachusetts) and the mid-Atlantic Bight are added as humpback whales may pass through these areas during migration (Fry 1988; Abend 1993; Abend and Smith 1997).

Differences in balaenopterid signature as a function of location and time of sample prompted an analysis of geographic, annual and species variation in prey samples. These analyses were limited owing to low sample sizes and lack of original data. However, geographic variation could be tested for capelin, herring and mackerel (J. Lawson and V. Lesage unpub. data; S. Todd unpub. data; W. Montevecchi and K. Hobson unpub. data; Abend and Smith 1997). Interannual variation was examined in capelin and herring (J. Lawson and V. Lesage unpub. data; S. Todd unpub. data; Dickson 1987; Hobson and Montevecchi 1991; Ostrom *et al.* 1993; Hobson *et al.* 1996). Finally, interspecies isotopic variability was tested in Arctic cod (*Boreogadus saida*), sand lance, shrimp (*Pandalus borealis*), squid (*Gonatus* sp.), capelin and herring (J. Lawson and V. Lesage unpub. data).

3.10 Developing an isotopic account of trophic levels for a Northwest Atlantic food web

Values of $\delta^{15}N$ can be used as a proxy for trophic level (TL), because of the ~3‰ enrichment factor per TL caused by fractionation. Alternatively, $\delta^{13}C$ values are less reliable in this estimation because of the smaller fractionation constant. Dickson (1987), Fry (1988) and Ostrom

Table 3.5. Species values of $\delta^{13}C$ and $\delta^{15}N$ ($\bar{X} \pm SD$ (‰)) for various potential prey items of Newfoundland balaenopterids. In some cases, ranges (in brackets) are also given, where available. Location abbreviations: SGB = Georges Bank (Massachusetts); BDE = Bay D'Espoir (GZ = 5); FB = Fortune Bay (GZ = 5); PB = Placentia Bay (GZ = 5); TB = Trinity Bay (GZ = 3); EC = East coast of Newfoundland, inshore (GZ = 3/4); ECO = East coast of Newfoundland, offshore; MAB = Mid-Atlantic Bight; GB = Grand Banks; NEO = Northeast coast of Newfoundland, offshore; GSL = Gulf of St. Lawrence; LN = Labrador coast, nearshore; LO = Labrador coast, offshore. A majority of generic and species names are standardized from Mauchline (1980), Scott and Scott (1988). For further information, see notes at end of table.

Common Name	Species	n	Location/When	$\bar{x} \pm SD$ (‰) $\delta^{13}C$	$\delta^{15}N$	Source
Zooplankton						
Euphausiids	not specified	7	SGB/86	-19.8 ± 0.4	7.9 ±0.5	Fry 1988
Euphausiid	unknown	1	PB/94	-19.7	13.3	S. Todd unpub. data
Euphausiids,	note (1)	19	BDE/84 – 85	-21.6 ±0.7	11.0 ±0.9	Dickson 1987
Decapods,				(-20.6 – -24.1)	(9.5 – 12.6)	
Amphipods	note (2)	40	FB/84 – 85	-22.4 ±0.9	11.3 ±1.4	" "
				(-21.1 – -23.7)	(9.7 – 14.4)	
Decapods	*Pandalus borealis*	10	ECO/95	-17.9 ± 0.25	11.3 ± 0.21	J. Lawson unpub. data
Copepods	note (3)	35	BDE/84 – 85	-22.6 ±0.8	10.0 ±1.3	Dickson 1987
				(-21.4 – -23.9)	(7.7 – 12.3)	
	note (4)	20	FB/84 – 85	-22.7 ±1.0	10.3 ±1.4	" "
				(-20.8 – -23.7)	(8.0 – 12.7)	
Ctenophores	not known	5	FB/84 – 85	-22.0 ±1.1	11.1 ±0.4	" "
	not known	3	BDE/84 – 85	-20.2 ±0.1	11.1 ±0.4	" "

(Table 2.5, continued)

Common Name	Species	n	Location/When	$\bar{X} \pm SD$ (‰) $\delta^{13}C$	$\delta^{15}N$	Source
Fish						
Sand Lance	*Ammodytes* sp.	4	SGB/86	-18.8 ±0.4	10.2 ±0.3	Fry 1988
		10	ECO/96	-19.2 ±0.26	14.1 ±0.39	J. Lawson unpub.data
Herring	*Clupea harengus*	1	BDE/84 – 85	-20.9	11.6	Dickson 1987
		1	SGB/86	-22.1	11.2	Fry 1988
		33	TB/91 – 92	-20.3 ±0.7	13.0 ±0.4	Hobson *et al.* 1996
		1	EC/94	-18.7	13.3	S. Todd unpub. data
		1	note (5)	-19.4	11.7	Abend 1993
		29	NEO,GSL/95-96	-19.9 ±0.88	13.4 ±0.55	J. Lawson and V. Lesage unpub. data
Capelin	*Mallotus villosus*	4	GB+EC/< 91	-	13.0 ±0.6	Hobson and Montevecchi 1991
		11	note (6)/86 – 90	-21.4 ±0.5	12.2 ±0.8	Ostrom *et al.* 1992
		3	FB/84 – 85	-22.5 ±1.4	11.7 ±0.2	Dickson 1987
		16	note (7)/94	-19.1 ±0.3	13.3 ±0.3	S. Todd unpub. data
		11	LN,GSL/95-96	20.1 ±0.87	13.9 ±0.71	J. Lawson and V. Lesage unpub. data

(Table 2.5, continued)

Common Name	Species	n	Location/When	$\bar{X} \pm SD$ (‰) $\delta^{13}C$	$\delta^{15}N$	Source
Fish (continued)						
Mackerel	*Scomber scombrus*	4	GB+EC/<91	-	12.9 ±0.8	Hobson and Montevecchi 1991
		9	MAB/91 – 92	-21.3 ±0.4	12.2 ±0.2	Abend and Smith 1995b
Arctic Cod	*Boreogadus saida*	10	ECO/96	-18.8 ±0.29	13.7 ±0.43	J. Lawson unpub. data
Squid						
unknown	-	2	SGB/86	-16.6 ±0.3	11.0 ±0.3	Fry 1988
Short-finned squid	*Gonatus* sp.	10	LO/96	-18.5 ±0.36	12.3 ±0.71	J. Lawson unpub. data
Short-finned squid	*Illex illecebrosus*	3	GB+EC/<91	-	11.9 ±0.1	Hobson and Montevecchi 1991
		2	note (8)/86 – 90	-20.2	15.1	Ostrom *et al.* 1992
		4	note (9)/86 – 90	-19.1 ±0.4	9.3 ±0.1	" "
Long-finned squid	*Loligo pealei*	9	MAB/91 – 92	-18.8 ±0.3	12.2 ±0.2	Abend and Smith 1997

Notes for Table 2.5:

(1) Species included (Euphausiids) *Meganyctiphanes norvegica, Thysanoessa inermis, T. raschii,* (Decapods) *Pasiphae multidentata, Sergestes arcticus,* (Amphipods) *Halirages fulvocinctus, Hyperia medusarum, Parathemisto abyssorum,* and *P. gaudichaudii.*

(2) Species included (Euphausiids) *Meganyctiphanes norvegica, Thysanoessa inermis, T. longicaudata, T. raschii,* (Decapods) *Pandalus borealis, Pandalus propinquis, Pasiphae multidentata,* (Amphipods) *Acanthostephia malmgreni, Halirages fulvocinctus, Hyperia medusarum, Parathemisto abyssorum,* and *P. gaudichaudii.*

(3) Species included *Calanus hyperboreus, Centropages hamatus, Euchaeta norvegica, Gaidius tenuispinus, Metridia longa, Pseudocalanus elongatus,* and *Temora longicornis.*

(4) Species included *Calanus hyperboreus, Centropages hamatus, Euchaeta norvegica, Metridia longa,* and *Temora longicornis.*

(5) Food taken from unspecified location used for captive subject, likely obtained before 1995.

(6) Unspecified location, but likely from nearshore Newfoundland.

(7) Sampled across all GZs.

(8) From Newfoundland nearshore waters, specified as 'large' (24.5 cm).

(9) From Newfoundland offshore waters (Grand Banks), specified as 'small' (14.5 ± 1 cm, mean length ± 1 s.d.).

et al. (1993) calibrated $\delta^{15}N$ values to trophic level (TL) for a range of species in Northwest Atlantic foodwebs.

Dickson (1987) and Fry (1988) calculated trophic enrichments for known predator-prey relationships, and averaged these on a per TL basis. Trophic enrichments were then added sequentially to the $\delta^{15}N$ value at the base of the foodweb (particulate organic matter suspended within the water column) to calculate the value of $\delta^{15}N$ per TL (Table 3.6). Ostrom *et al.* (1993) used a known herbivore as their foodweb base, and calculated TL of sampled consumers relative to that herbivore on the assumption that enrichment per trophic level is 3‰. For this study, values for $\delta^{15}N$ for each TL were calculated by taking the average of data relevant to a Newfoundland-based ecosystem (Table 3.6; Dickson 1987; Ostrom *et al.* 1993).

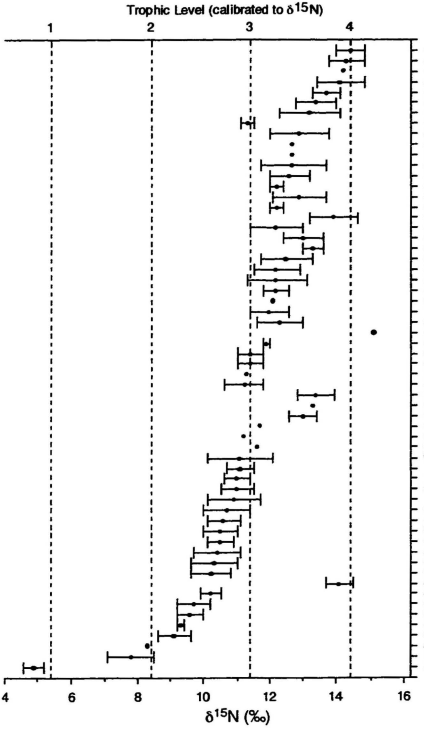
Average species $\delta^{15}N$ values (Table 3.5), with standard deviations where available, are plotted in Figure 3.4, along with a calibrated TL scale (Table 3.6). This diagram shows good general agreement with that composed by Fry (1988), although absolute figures differ (likely due to differences in the phytoplankton base of either food chain). The lower end of the trophic map is dominated by small zooplankton such as copepods, moving up to ctenophores and medusans, euphausiids and amphipods, squid and planktivorous fish, and finally piscivores.

Some species values should be considered with caution; for example, the lower of two $\delta^{15}N$ values for sand lance (*A. americanus*) comes from a trophic map that has consistently lower $\delta^{15}N$ values (Fry 1988). In some instances several estimates of a species' isotopic composition are available. In such cases, species means are in general agreement, being within approximately 2‰ of each other and within the same trophic level (e.g., *M. villosus*, *C. harengus*, *S. scombrus*). Variability within species measurements can be expected as a function of location sampled, age and sex of animal (Gearing 1991). For example, $\delta^{15}N$ values for the short-finned squid (*I. illecebrosus*) are highly variable, with a range of 5.8‰ (Hobson and Montevecchi 1991; Ostrom *et al.* 1993). This can be explained by the different lengths of animal sampled (range = 14.5–24.5 cm), which might affect the types of prey that could be caught by

Table 3.6. Values of $\delta^{15}N$ on a per trophic level (TL) basis for three studies based in the Northwest Atlantic. Values of TL given by Ostrom *et al.* (1992) have been shifted by down by 1 TL to standardize the base used in each trophic web. Average TL is calculated on the basis of data relevant only to Newfoundland waters (Dickson 1987; Ostrom *et al.* 1992).

		Values of $\delta^{15}N$ (‰)			Average
		Dickson (1987)	Fry (1988)	Ostrom *et al.* (1992)	$\delta^{15}N$ used
TL Enrichment (f_N)		3.1	3.6	3.0	3.0
TL	1	4.9	-	5.8	5.4
	2	8.0	5.8	8.8	8.4
	3	11.1	9.4	11.8	11.4
	4	14.2	13.0	14.8	14.4
	5	17.3	16.6	18.8	17.4

Figure 3.4. Mean values of $\delta^{15}N$ for various potential prey and other species in the Northwest Atlantic (error bars indicate ±1 *SD*), calibrated by trophic level (Table 3.6), and modelled after a schematic by Fry (1988). Values taken from Dickson (1987), except (2) Fry (1988), (3) Abend and Smith (1993), (4) Hobson *et al.* (1996), (5) S. Todd (unpub. data), (6) Hobson and Montevecchi (1991), (7) Ostrom *et al.* (1993), (8) J. Lawson (unpub. data), and (9) J. Lawson and V. Lesage (unpub. data). POM = Particulate Organic Matter, suspended in the water column, sampled at a depth of 5 m.


Trophic Level (calibrated to δ15N)
1
2
3
4
Sagitta maxima
Melanostigma atlanticum
Sebastes sp.
Acanthostephia malgremi
Boreogadus saida (8)
Boreomysis nobilis
Pandalus borealis
Pandalus borealis (8)
Benthosema glaciale
Pseudomma truncatum
Pandalus propinquis
Sagitta elegans
Pasiphaea multidentata
Loligo pealei (3)
Scomber scombrus (6)
Scomber scombrus (3)
Mallotus villosus (9)
Mallotus villosus (7)
Mallotus villosus (6)
Mallotus villosus (5)
Mallotus villosus
Sergestes arcticus
Euchaeta norvegica
Eutrichnia hamata
Cydothone microdon
Thysanoessa inermis
Gonatus sp.
Illex illecebrosus (7)
Illex illecebrosus (6)
Boreomysis arctica
Hyperia medusarum
Parathemisto abyssorum
Melerythrops robusta
Clupea harengus (9)
Clupea harengus (5)
Clupea harengus (4)
Clupea harengus (3)
Clupea harengus (2)
Clupea harengus
Ctenophores
Thysanoessa raschii
Tomopteris helgolandica
Mysis mixta
Thysanoessa longicaudata
Gaidius tenuispinus
Parathemisto gaudichaudi
Ostracods
Glyptocephalus cynoglossus
Metridia longa
Halirages fulvocinctus
Clione limacina
Ammodytes americanus (8)
Ammodytes americanus (3)
Meganyctiphanes norvegica
Calanus hyperboreus
Illex illecebrosus (7)
Centropages hamatus
Aurelia sp.
Temora longicornis
POM
4
6
8
10
12
14
16
δ15N (‰)

the animal, and therefore potentially impacting upon its $\delta^{15}N$ value. Other groups (e.g., crustaceans) also display high isotopic variability between species, likely a function of a lack of standardization between SIA studies, as well as differences in trophic level (Dickson 1987).

3.11 Balaenopterid feeding within a Northwest Atlantic food web

If balaenopterid isotopic signatures reflect assimilated prey, then humpback values $\delta^{15}N$ and $\delta^{13}C$ should vary as a function of factors that also affect prey availability. Two such factors would include location and time of sampling, variables which may therefore potentially confound the data collected in this study. One method to isolate such confounding effects is to examine data for each combination of year and GZ sampled, thus avoiding variability across those variables. To do this, isotopic levels were considered as a function of DoY, for each combination of year and GZ treatment levels. Availability of major prey items was thought to be the primary controlling factor in determining consumer isotopic levels, and thus dates of capelin spawning and peak abundance were superimposed upon these data (B. Nakashima unpub. data; Nakashima and Winters 1995).

It was expected that balaenopterid isotopic levels would vary as a function of DoY because of prey availability. This hypothesis was tested using linear regression of DoY on CIG, $\delta^{15}N$ and $\delta^{13}C$, for each combination of year and GZ. However, the assumption of linearity is inappropriate, because humpbacks feed upon several prey species that vary both in isotopic signature and time of peak abundance (Table 3.5, Figure 3.4; Nakashima 1995). Thus, the resulting humpback consumer signature may not be linear, but instead a complex waveform that reflects species composition of prey taken as a function of time; signatures examined longitudinally would be expected to exhibit a series of peaks that correspond to prey availability (Schell and Saupe 1993; Ames *et al.* 1996; Hobson and Stirling 1997). Furthermore, the function describing humpback isotopic signature would be shifted in time relative to prey

availabilities to account for integration time of the prey's signature into the tissues sampled in the consumer (Tieszen *et al.* 1983; Hobson and Clark 1992a).

A statistical definition of such a function is difficult owing to its complexity. Instead, qualitative curve smoothing was used to identify possible trends in consumer isotopic signatures, by employing a running average of three consecutive data points.

4. Results - Analysis of stable isotopes measured in balaenopterids

4.1 Analysis of humpback whale samples

Overall, humpback whales had a $\delta^{13}C$ value ($\bar{x} \pm SD$) of -18.5 ± 0.28‰ (n = 98) and a $\delta^{15}N$ value of 14.6 ± 0.94‰ (n = 106). Nitrogen isotopic variability ($CV \pm SE_{CV}$ = 6.4 ± 0.44%) was higher than carbon isotopic variability (1.50 ± 0.11%), by a factor of approximately four. Average percent lipid composition of humpback skin was 9.8 ± 2.22% by mass (n = 50).

4.1.1 Sexual differences

CIG was not significantly predicted by sex (p = 0.12; Figure 4.1). There were no sexual differences in carbon or nitrogen isotopic levels (p = 0.08 and 0.86, respectively), but female skin had a significantly higher lipid content than male skin (p = 0.01; Table 4.1; Figure 4.2).

4.1.2 Geographic variation

Geographic variation (by GZ) was not be detected when either $\delta^{13}C$ or $\delta^{15}N$ variables were examined separately (p = 0.08 and 0.07, respectively; Table 4.2). When GZs were grouped with respect to oceanographic considerations, a significant difference was seen between the North/East Coast and the Southern Shore/South Coast groupings with respect to $\delta^{13}C$ (p = 0.02), and $\delta^{15}N$ values (p = 0.05; Table 4.2). CIG also varied significantly by location sampled (p = 0.01; Table 4.2; Figure 4.3). CIG means that were isotopically similar tended to be from areas that were in close physical proximity; for example, the East Coast (GZ4) and the South

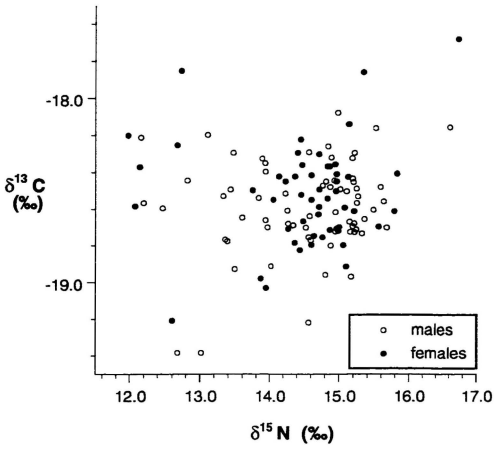


Figure 4.1. Humpback whale $\delta^{13}C$ and $\delta^{15}N$ paired isotopic values as a function of sex. No separation of CIG by sex was apparent.

Table 4.1. Humpback whale isotopic and lipid differences by sex. Males and females were different only in percentage lipid contribution to the skin biopsy.

	Males		Females			
Variable and test type	n	$\bar{X} \pm SD$	n	$\bar{X} \pm SD$	Test statistic	Significance
CIG, Wilk's	-	-	-	-	$\lambda = 0.89$	*ns*
$\delta^{13}C$, *t*-test	55	-18.6 ± 0.27‰	43	-18.5 ± 0.29‰	$t = 1.78$	*ns*
$\delta^{15}N$, Mann-Whitney	57	14.5 ± 1.00‰	49	14.6 ± 0.87‰	$U = 1362.00$	*ns*
%lipid, z-test	23	9.2 ± 1.69%	26	10.5 ± 2.43%	$z = 2.24$	***

ns = not significant, *$p < 0.05$, **$p < 0.025$, ***$p < 0.01$.

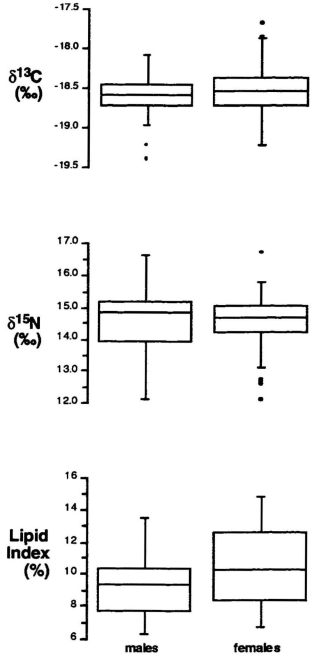


Figure 4.2. Values of $\delta^{13}C$ and $\delta^{15}N$ (‰, outliers removed), and lipid content (% by mass) for humpback whales sampled between 1992-1994. No sexual differences existed for either isotope. Female skin biopsies had higher lipid content than male skin biopsies.

Table 4.2. Isotope values ($\bar{X} \pm SD$) for humpback whales sampled 1992-1994, reported a) for each geographic zone (GZ), and b) for combined GZs. No geographic variation was seen in either isotopic variable considered separately. However, considered together, CIGs were predicted by GZ. When grouped according to oceanographic factors (Table 4.2b), significant differences were seen in both isotopic variables.

a)

		$\delta^{13}C$ (‰)		$\delta^{15}N$ (‰)	
GZ	CIG	*n*	$\bar{X} \pm SD$	*n*	$\bar{X} \pm SD$
1	-	8	-18.4 ± 0.23	8	14.7 ± 1.06
2	-	20	-18.6 ± 0.24	20	14.2 ± 0.91
3	-	17	-18.4 ± 0.23	24	14.3 ± 1.03
4	-	33	-18.5 ± 0.30	34	14.8 ± 0.91
5	-	20	-18.6 ± 0.30	20	14.8 ± 0.73
Test type	Wilk's		ANOVA		Kruskal-Wallis
Test statistic	$\lambda = 0.82$		$F = 2.18$		$H = 8.68$
Significance	***		*ns*		*ns*

ns = not significant, $^{*}p < 0.05$, $^{**}p < 0.025$, $^{***}p < 0.01$.

b)

	North/East Coast		South. Shore/South Coast		Test type	Test Statistic	Signif.
	n	$\bar{X} \pm SD$ (‰)	*n*	$\bar{X} \pm SD$ (‰)			
$\delta^{13}C$	25	-18.4 ± 0.23	53	-18.6 ± 0.30	ANOVA	$F = 5.40$	**
$\delta^{15}N$	33	14.4 ± 1.03	54	14.8 ± 0.84	ANOVA	$F = 2.58$	*

ns = not significant, $^{*}p < 0.05$, $^{**}p < 0.025$, $^{***}p < 0.01$.

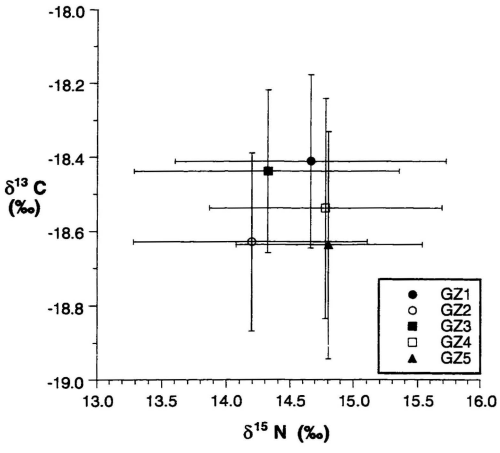


Figure 4.3. Separation of CIGs by geographic zone (GZ) for humpback whales. Error bars represent ± 1 *SD*. Variation by GZ was not significant by either isotopic variable separately. However, when considered together, isotopic differences between GZs can be shown. Largest differences are between the North Coast and either Labrador (GZ1) or the South Coast (GZ5), and Bonavista/Trinity Bay (GZ3) and the South Coast (GZ5). Closest similarities are between the East Coast (GZ4) and the South Coast (GZ5), the North Coast (GZ1) and either Bonavista/Trinity Bay (GZ3) or the East Coast/Southern Shore (GZ4).

Coast (GZ5), the North Coast (GZ1) and either Bonavista/Trinity Bay (GZ3) or the East Coast/Southern Shore (GZ4; Figure 4.3). CIG means that were isotopically disimilar tended to be from areas that were separated by the greatest geographic distance (for example, Bonavista/Trinity Bay and the South Coast, or the North Coast and the South Coast). The one exception was the large CIG means difference between the North Coast and the West Coast/Labrador. However, it should be noted that the largest isotopic difference seen geographically (that is, between GZ1 and GZ2), was still relatively small ($\Delta\delta^{15}N$, ~0.5‰; $\Delta\delta^{13}C$, ~0.2‰).

Values of $\delta^{13}C$ could not be predicted by knowledge of latitude (p =0.60) or longitude (p = 0.71; Table 4.3). Values of $\delta^{15}N$ were significantly negatively correlated with latitude ($p <$ 0.01; Figure 4.4), but not longitude (p = 0.09; Table 4.3). CIG could not be predicted by knowledge of latitude (p = 0.08) or longitude (p = 0.76; Table 4.3).

4.1.3 Seasonal variation

Stable-carbon values ($\delta^{13}C$) did not vary by month, in any year ($p \geq 0.16$ for all cases). Significant differences in $\delta^{15}N$ values as a function of month were seen in 1992 and 1993, but not 1994 (p = 0.02, 0.05, and 0.45, respectively; Table 4.4; Figure 4.5). Values of $\delta^{15}N$ tended to peak in July, with consistently lower values in August and September (Mann-Whitney's U; $p \leq 0.01$ for either 1992 or 1993). Monthly differences in CIG were only found in 1993 (p = 0.02; Table 4.4; Figure 4.5a). The greatest difference in CIG occurred between June and either July or August.

No significant relationship between Day of Year (DoY) and either $\delta^{13}C$ or $\delta^{15}N$ was observed for any year ($p \geq 0.07$ for all cases; Table 4.4b). However, DoY was significantly correlated with CIG in 1993, but not in 1992 or 1994 (p = 0.01, 0.15, and 0.30, respectively; Table 4.4b).

Table 4.3. Prediction of isotopic values by latitude and longitude using regression. Only latitude and values of $\delta^{15}N$ were significantly correlated, suggesting that $\delta^{15}N$ values decreased with northward movement.

Variable (y)	Treatment (x)	Test type	n	Test statistic	Significance	Predictive eq'n (if significant)
CIG	Latitude	Wilk's	98	$\lambda = 0.95$	*ns*	-
	Longitude			$\lambda = 0.99$	*ns*	-
$\delta^{13}C$	Latitude	Pearson	98	$t = -0.52$, $r^2 < 0.00$	*ns*	-
	Longitude			$t = -0.37$, $r^2 < 0.00$	*ns*	-
$\delta^{15}N$	Latitude	Spearman	106	$z = 3.31$, $\rho = -0.30$	***	$y' = 20.27 - 0.12x$
	Longitude			$z = 1.69$, $\rho = -0.17$	*ns*	-

ns = not significant, $^{*}p < 0.05$, $^{**}p < 0.025$, $^{***}p < 0.01$.

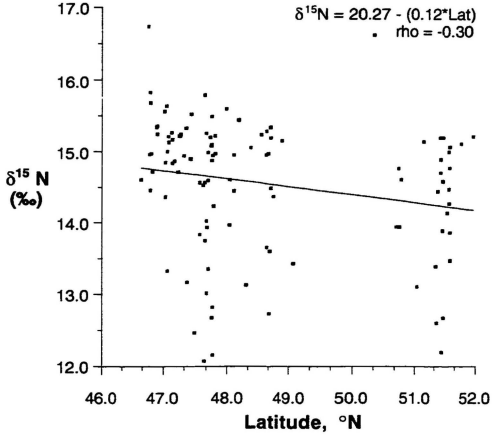


Figure 4.4. Relationship of $\delta^{15}N$ to latitude of sighting. Values of $\delta^{15}N$ significantly decreased in northern sampling locations.

Table 4.4. Intra-annual isotopic variation; a) means and standard deviations, and b) statistical tests (K-W = Kruskal-Wallis). Monthly differences in $\delta^{15}N$ were evident for 1992 and 1994, reflected by an increase in July relative to August and June. August and September values were consistently lower than June and July values. Differences in CIG were only detected in 1993, either by month or by Day of Year (DoY).

a)

		June		July		August		September	
Year		n	$\bar{X} \pm SD$ (‰)	n	$\bar{X} \pm SD$ (‰)	n	$\bar{X} \pm SD$ (‰)	n	$\bar{X} \pm SD$ (‰)
1992	$\delta^{13}C$	8	-18.5 ± 0.23	8	-18.6 ± 0.30	10	-18.8 ± 0.32	9	-18.6 ±0.20
	$\delta^{15}N$		14.3 ± 1.07	11	15.0 ± 0.90		14.0 ± 0.74		14.1 ± 1.01
1993	$\delta^{13}C$	10	-18.7 ± 0.29	16	-18.5 ± 0.24	14	-18.5 ± 0.30	-	-
	$\delta^{15}N$		14.6 ± 0.89		14.9 ± 0.48		14.1 ± 0.91		-
1994	$\delta^{13}C$	7	-18.6 ± 0.11	12	-18.4 ± 0.27	4	-18.4 ± 0.39	-	-
	$\delta^{15}N$		15.0 ± 0.78		15.0 ± 1.13	9	14.5 ± 0.96		-
Pooled	$\delta^{13}C$	25	-18.6 ± 0.24	36	-18.5 ± 0.27	28	- 18.6 ± 0.34	9	-18.6 ±0.20
	$\delta^{15}N$		14.6 ± 0.93	39	15.0 ± 0.82	33	14.2 ± 0.88		14.1 ± 1.01

b)

Year		Month			DoY		
		Test type	Test statistic	Signif.	Test type	Test statistic	Signif.
1992	CIG	Wilk's	$\lambda = 0.68$	*ns*	Wilk's	$\lambda = 0.89$	*ns*
	$\delta^{13}C$	ANOVA	$F = 1.85$	*ns*	Pearson	$F = 2.54$, $r = -0.07$	*ns*
	$\delta^{15}N$	K-W	$H = 9.48$	**	Spearman	$z = -1.82$, $\rho = -0.30$	*ns*
1993	CIG	Wilk's	$\lambda = 0.71$	**	Wilk's	$\lambda = 0.79$	**
	$\delta^{13}C$	ANOVA	$F = 1.77$	*ns*	Pearson	$F = 2.45$, $r = -0.06$	*ns*
	$\delta^{15}N$	K-W	$H = 6.18$	*	Spearman	$z = -1.41$, $\rho = -0.23$	*ns*
1994	CIG	Wilk's	$\lambda = 0.74$	*ns*	Wilk's	$\lambda = 0.89$	*ns*
	$\delta^{13}C$	ANOVA	$F = 1.44$	*ns*	Pearson	$F = 1.19$, $r = -0.05$	*ns*
	$\delta^{15}N$	K-W	$H = 1.46$	*ns*	Spearman	$z = -1.02$, $\rho = -0.20$	*ns*
Pooled	CIG	Wilk's	$\lambda = 0.79$	***	Wilk's	$\lambda = 0.90$	***
(no adj.)	$\delta^{13}C$	ANOVA	$F = 1.31$	*ns*	Pearson	$F = 0.05$, $r < 0.00$	*ns*
	$\delta^{15}N$	K-W	$H = 17.33$	***	Spearman	$z = -3.09$, $\rho = -0.30$	***
Pooled	CIG	-	-	-	Wilk's	$\lambda = 0.90$	***
(spawn)	$\delta^{13}C$	-	-	-	Pearson	$F = 0.13$, $r < 0.00$	*ns*
	$\delta^{15}N$	-	-	-	Spearman	$z = -3.03$, $\rho = -0.30$	***
Pooled	CIG	-	-	-	Wilk's	$\lambda = 0.90$	***
(peak)	$\delta^{13}C$	-	-	-	Pearson	$F = 0.15$, $r < 0.00$	*ns*
	$\delta^{15}N$	-	-	-	Spearman	$z = -3.04$, $\rho = -0.30$	***

ns = not significant, $*p < 0.05$, $**p < 0.025$, $***p < 0.01$.

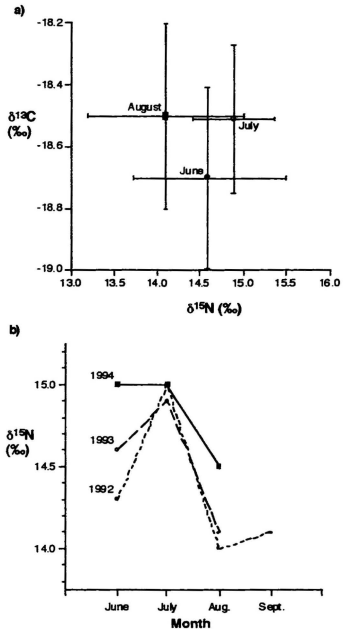


Figure 4.5. Intra-annual variation in specific years: a) CIGs for 1993; and b) $\delta^{15}N$ variation by month. Error bars indicate ± 1 *SD*. Greatest difference between CIGs in 1993 occurred between June and the remaining two months. Levels of $\delta^{15}N$ typically peaked in July, with August having consistently lower values than June.

Monthly variation in $\delta^{13}C$ was not significant for pooled years, in contrast to monthly variation in $\delta^{15}N$, which was significant ($p = 0.28$ and < 0.01, respectively; Table 4.4; Figure 4.6a). Qualitatively, both isotopic variables demonstrated the same monthly trends, although the $\delta^{15}N$ variable was more exaggerated; specifically, isotopic ratios started in June at some intermediate level, rose to a peak in July, and decreased to a minimum in August (Mann-Whitney's U, $p < 0.01$; Figure 4.6a). Significant monthly differences in CIG also existed when years were pooled ($p < 0.01$; Table 4.4; Figure 4.6b).

To examine the relationship of isotopic value to DoY for pooled data, years were synchronized by two methods outlined in Section 3.7 (either by peak spawning or peak abundance of capelin). Values of $\delta^{13}C$ were not related to DoY for spawning adjusted or peak-adjusted years (Section 3.7). However, $\delta^{15}N$ values were significantly negatively related to either DoY variable ($p < 0.01$ for both spawning adjusted or peak-adjusted years; Table 4.4; Figure 4.7), reflecting the similar decrease in either isotopic ratio beween July and August. CIG was significantly related to DoY pooled across synchronized years ($p < 0.01$ for either method of synchronization; Table 4.4).

4.1.4 Individual variation within seasons

For the eight individuals that were resampled in 1992, isotopic composition did not change within the resampling interval ($\delta^{13}C$, $t = 0.20$, $p = 0.85$; $\delta^{15}N$, $t = 0.25$, $p = 0.81$). However, this result is due to inconsistent direction of change within each variable across individuals, and inconsistent resampling intervals (Figure 4.8a). In fact, per individual, some large changes in isotopic composition were seen, as high as 3‰ ($\delta^{15}N$, EC92114). Specifically, six of the eight resampled individuals demonstrated changes in one isotopic variable that were paralleled by the other isotopic variable (Figure 4.8a). Of the remaining three, isotopic ratios shifted in opposite directions or remained constant. Two animals showed increasing $\delta^{13}C$ and $\delta^{15}N$ values

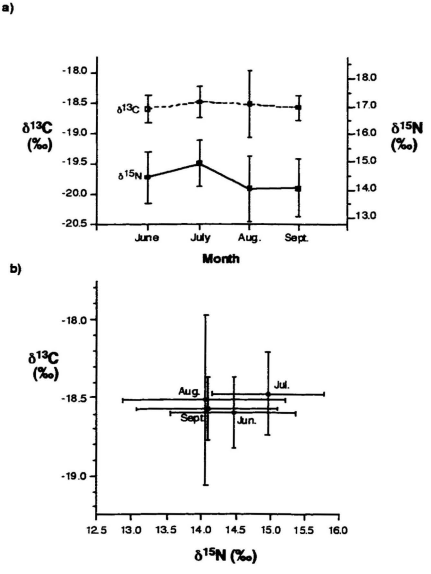
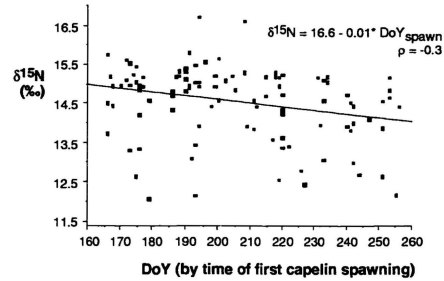


Figure 4.6. Humpback whale intra-annual isotopic variation for pooled years: a) monthly means for $\delta^{13}C$ and $\delta^{15}N$, and b) variation in CIG by month. Error bars indicate ±1 *SD*. Most variability occured within the $\delta^{15}N$ variable. Monthly trends in variability were similar for either isotopic variable, although differences between monthly $\delta^{15}N$ means were greater than their $\delta^{13}C$ counterparts. Greatest difference in CIG occurred between July and both August or September (Mann-Whitney's U, $p < 0.01$ for either pairwise comparison).

a)



b)

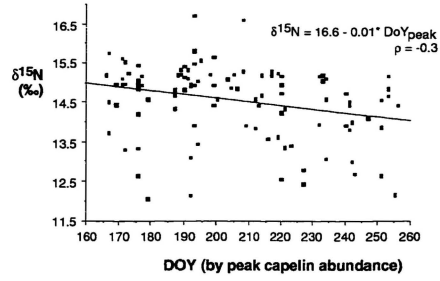


Figure 4.7. Humpback whale intra-annual $\delta^{15}N$ variation as a function of Day of Year (DoY) for pooled years 1992-1994: a) synchronizing years by first spawning of capelin, and b) synchronizing years by peak abundance of capelin. Both methods of synchronization demonstrate a significant negative linear relationship between $\delta^{15}N$ and DoY.

a)

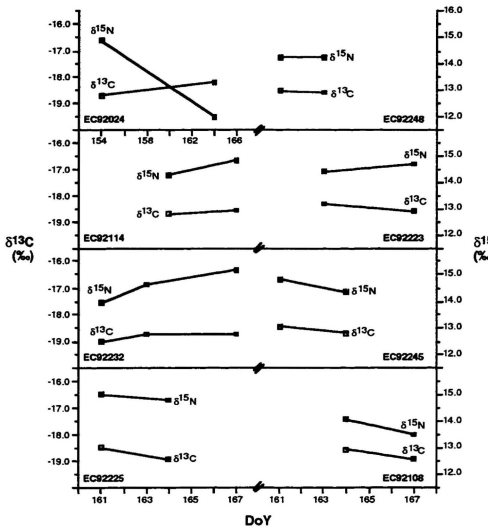
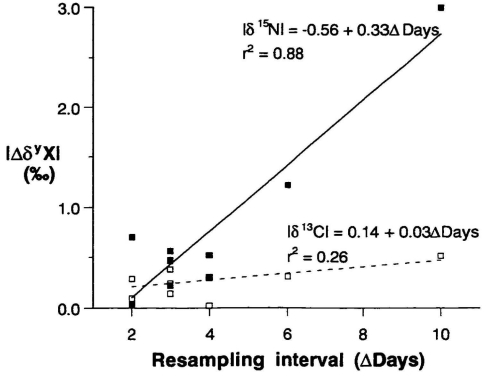


Figure 4.8. Isotopic composition for eight resampled individuals biopsied on different days of the year (DoY) in 1992, showing: a) changes in $\delta^{13}C$ and $\delta^{15}N$ for each individual; and b) isotopic variability within individuals as a function of resampling interval. All animals were biopsied in Witless Bay (Figure 3.1). In Figure 4.8b, isotopic variability is expressed as an absolute change in isotopic value between two resampling events, and plotted against the interval between resamplings. Absolute changes in $\delta^{15}N$ were significantly related to sampling interval, although absolute changes in concurrent $\delta^{13}C$ readings were not.

b)


3.0
2.0
1.0
0.0
|Δδ^YX|
(‰)
|δ 15N| = -0.56 + 0.33Δ Days
r2 = 0.88
|δ 13C| = 0.14 + 0.03ΔDays
r2 = 0.26
2
4
6
8
10
Resampling interval (ΔDays)

(EC92114; EC92232) with $\delta^{15}N$ values (~0.20‰.day^{-1}) increasing ~four times greater than $\delta^{13}C$ values (~0.05‰.day^{-1}). Both individuals started at similar values for both isotopes. Three animals showed decreasing $\delta^{13}C$ and $\delta^{15}N$ values (EC92245, EC92225, EC92108), declining at variable rates for either $\delta^{13}C$ values (-0.79 – -0.16‰.day^{-1}) or $\delta^{15}N$ (-0.72 – -0.19‰.day^{-1}). Individual EC92024 displayed decreasing $\delta^{15}N$ values, yet increasing $\delta^{13}C$ values (Figure 4.8a). This individual also showed the largest decreasing rate for $\delta^{15}N$, dropping to a value that seems unusually low for humpback whales ($\delta^{15}N$ = 11.9‰). The remaining animal, EC92223, demonstrated increasing $\delta^{15}N$ values, yet decreasing $\delta^{13}C$ values (Figure 4.8a).

The magnitude of change in isotopic composition as a function of sampling interval is shown in Figure 4.8b. Absolute changes in $\delta^{15}N$ were significantly related to sampling interval (r^2 = 0.88, $p < 0.01$). Absolute changes in concurrent $\delta^{13}C$ readings were not significantly related to sampling interval. Variability in $\delta^{13}C$ values was less than 0.5‰ over a 10 day sampling interval. The gradient of change for $\delta^{13}C$ values was approximately one third that for $\delta^{15}N$ values, a factor equivalent to the fractionation difference observed between the isotopic variables in term of trophic enrichment (cf. 1‰ and 3‰ respectively).

4.1.5 Interannual variation

For the period 1992-1994, no interannual variation in $\delta^{13}C$ was seen, although levels of $\delta^{15}N$ changed significantly (p = 0.07 and 0.05, respectively; Table 4.5a; Figure 4.9). Mean $\delta^{15}N$ for 1994 was significantly higher that the annual mean for 1992 ($p < 0.01$; Table 4.5b). Significant yearly differences in CIG were also observed (p = 0.04; Table 4.5a; Figure 4.9)

Long-term trends were confirmed by the analysis of additional data from other years (P. Ostrom and J. Lien unpub. data). In this expanded dataset (1988-1994), significant interannual differences were seen in all isotopic variables ($p < 0.01$ for all analyses; Table 4.6a; Figure

Table 4.5. Interannual variability in CIG, and $\delta^{13}C$ and $\delta^{15}N$ separately for YoNAH years: a) means, standard deviations and statistics, and b) p-levels for Mann-Whitney's *U* pairwise tests on $\delta^{15}N$ annual means (using $\alpha = 0.01$ to protect against *post hoc* comparisons; significant values are **bolded**). K-W = Kruskal-Wallis. Nitrogen isotopic-ratios significantly increased from 1992 to 1994.

a)

	1992		1993		1994		Type		
	n	$\bar{X} \pm SD$ (‰)	*n*	$\bar{X} \pm SD$ (‰)	*n*	$\bar{X} \pm SD$ (‰)	of test	Test	Signif.
$\delta^{13}C$	54	-18.6 ± 0.26	41	-18.6 ± 0.28	23	-18.5 ± 0.27	ANOVA	$F = 2.59$	*ns*
$\delta^{15}N$	57	14.3 ± 0.94	41	14.6 ± 0.81	28	14.9 ± 0.99	K-W	$H = 9.36$	***
CIG		-		-		-	Wilks	$\lambda = 0.92$	*

ns = not significant, $^{*}p < 0.05$, $^{**}p < 0.025$, $^{***}p < 0.01$.

b)

	p-levels		
Year	1992	1993	1994
1993	0.193	-	-
1994	**< 0.01**	0.027	-

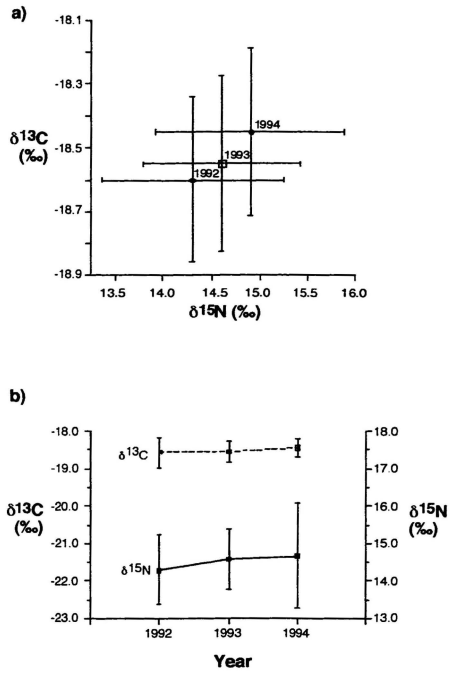


Figure 4.9. Interannual isotopic variability for 1992-1994, in a) CIG, and b) $\delta^{13}C$ and $\delta^{15}N$ isotopic ratios. Error bars give ± 1 *SD*. Isotopic variables increased from 1992 to 1994 with respect to both $\delta^{13}C$ and $\delta^{15}N$. Greatest increases were seen in the $\delta^{15}N$ variable.

Table 4.6. Interannual variability for YoNAH and non-YoNAH years, showing a) means, standard deviations and statistics, and b) pairwise comparisons for annual means of $\delta^{13}C$ (using Scheffé tests) and $\delta^{15}N$ (using Mann-Whitney's U at $\alpha = 0.01$; significant values are **bolded**). Data from 1988 ($n = 1$) are omitted from further statistical analyses.

a)

		$\bar{x} \pm SD$ (‰)		
Year	n	$\delta^{13}C$	$\delta^{15}N$	CIG
1988	1	-17.7	13.5	
1990	8	-17.6 ±0.41	13.4 ±1.04	
1991	3	-18.2 ±0.30	14.1 ±0.59	
1992	54	-18.6 ±0.26	14.3 ±0.94	
1993	41	-18.6 ±0.28	14.6 ±0.81	
1994	23	-18.5 ±0.27	14.9 ±0.99	
Test type		ANOVA	K-W	Wilk's
Test statistic		$F = 18.73$	$H = 24.28$	$\lambda = 0.50$
Significance		***	***	***

ns = not significant, $^{*}p < 0.05$, $^{**}p < 0.025$, $^{***}p < 0.01$.

b)

	$\delta^{13}C$					$\delta^{15}N$				
Year	1990	1991	1992	1993	1994	1990	1991	1992	1993	1994
1991	0.24	-	-	-	-	0.16	-	-	-	-
1992	< 0.01	0.22	-	-	-	0.02	0.22	-	-	-
1993	< 0.01	0.35	0.98	-	-	**< 0.01**	0.09	0.05	-	-
1994	< 0.01	0.72	0.44	0.82	-	**< 0.01**	0.03	**< 0.01**	0.02	-

4.10). *Post hoc* testing (Table 4.6b) confirmed a steady linear increase in $\delta^{15}N$ isotopic ratios since 1988 ($r^2 = 0.12$, $p < 0.01$; Figure 4.11a); concurrently, $\delta^{13}C$ isotopic ratios decreased. With respect to the latter, this decrease had both linear and quadratic components ($r^2_{lin.} = 0.11$, $p < 0.01$; $r^2_{quad.} = 0.31$, $p < 0.01$; Figure 4.11b).

4.1.6 Comparison of humpback whales with other balaenopterids

Finbacks had significantly lower $\delta^{15}N$ values than humpback or minke whales ($p < 0.01$ for either species), and significantly higher $\delta^{13}C$ values than humpbacks ($p < 0.01$; Table 4.7; Figure 4.12). The variability in $\delta^{13}C$ values for finbacks ($CV_{finback} \pm SE_{CV} = 1.3 \pm 0.40\%$) was comparable to that of humpbacks ($1.50 \pm 0.11\%$). However, the variability in $\delta^{15}N$ values of finbacks ($14.6 \pm 4.71\%$) was higher than that of humpbacks ($6.4 \pm 0.44\%$). Humpbacks and finbacks could also be separated on the basis of CIG ($p < 0.01$; Table 4.7; Figure 4.12).

In addition to isotopic values, species could also be distinguished on basis of the lipid content in the skin biopsy: finbacks had significantly more lipids by mass than humpbacks ($p < 0.01$; Table 4.7). The minke whale did not have a significantly different lipid content from either finbacks or humpbacks ($p > 0.05$; Table 4.7).

4.2 Analysis of prey samples

Capelin sampled showed no isotopic variation across GZs, for either CIG, $\delta^{13}C$ or $\delta^{15}N$ variables ($p = 0.79$, 0.21, and 0.08, respectively; Table 4.8a; S. Todd unpub. data; J. Lawson and V. Lesage unpub. data). Isotopic ratios also did not vary with longitude or latitude ($\delta^{13}C$, $r^2 = 0.48$, $p = 0.28$; $\delta^{15}N$, $r^2 = 0.12$, $p = 0.37$; S. Todd unpub. data).

No isotopic differences existed between capelin taken from the inshore waters of Labrador and the Gulf of St. Lawrence ($\delta^{13}C$, $p = 0.10$; $\delta^{15}N$, $p = 0.07$). Similarly, no isotopic differences

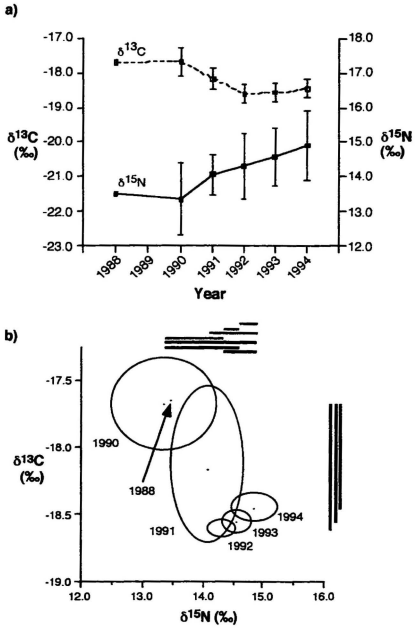


Figure 4.10. Humpback whale interannual isotopic variation for 6 years: a) annual $\delta^{13}C$ and $\delta^{15}N$ means, and b) annual CIGs. Ellipses represent 95% confidence intervals for axes of interest. Data from 1988 (+) consist of one point, and therefore have no associated ellipse. Bars indicate significant differences between means at $\alpha = 0.01$ and $\alpha = 0.05$ (solid and grey bars respectively). Error bars represent $\pm 1\,SD$.

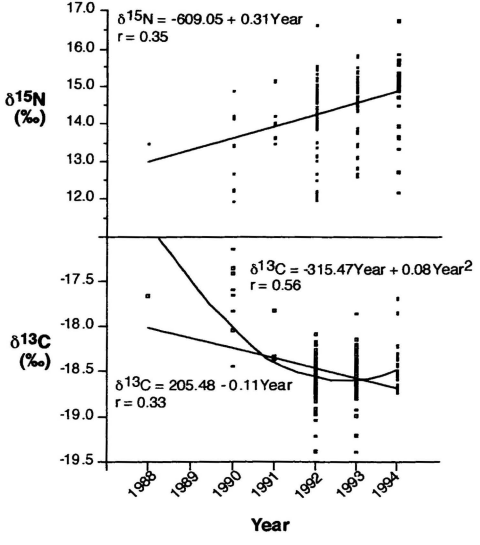


Figure 4.11. Humpback whale $\delta^{15}N$ and $\delta^{13}C$ isotopic values by year. Yearly values of $\delta^{15}N$ increase linearly. Yearly values of $\delta^{13}C$ show both negative linear and positive quadratic components.

Table 4.7. Interspecies isotopic variation for balaenopterids: a) means and standard deviations for humpback (Mn), finback (Bp) and minke whales (Ba); and b) statistical tests of differences between species, using Mann-Whitney's *U* between humpbacks and finbacks, and one-sample *z*-tests between minke whales and other species. Lipid contents were also compared using *z*-tests.

a)

		$\bar{x} \pm SD$		
Species	*n*	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	Lipid (%)
Megaptera novaeangliae (Mn)	120	-18.5 ±0.36	14.4 ±0.96	9.9 ± 2.19
Balaenoptera physalus (Bp)	5	-18.2 ±0.22	10.9 ±1.60	21.2 ± 10.84
B. acutorostrata (Ba)	1	-	15.5	5.1

b)

	$\delta^{13}C$		$\delta^{15}N$		CIG		Lipid	
	Mn	Bp	Mn	Bp	Mn	Bp	Mn	Bp
Bp	$U = 137.0$	-	$U = 13.0$	-	$\lambda = 0.69$	-	$z = 6.83$	-
	**		***		***		***	
Ba	-	-	$z = 0.97$	$z = 2.87$	-	-	$z = 0.16$	$z = 0.37$
			ns	***			*ns*	*ns*

ns = not significant, $^{*}p < 0.05$, $^{**}p < 0.025$, $^{***}p < 0.01$.

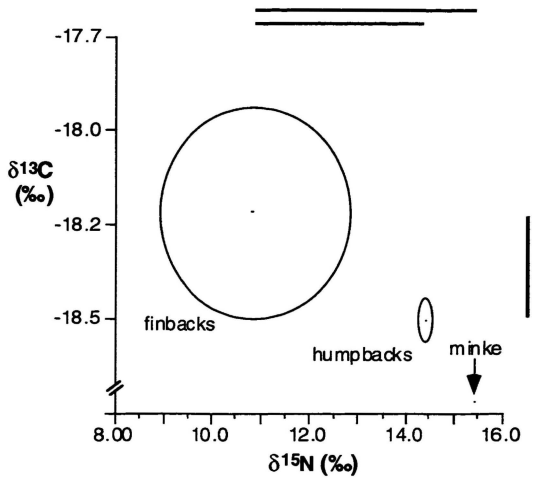


Figure 4.12. Interspecies variation for balaenopterids by CIG. Ellipses represent 95% confidence intervals. Solid bars represent differences between means at $\alpha = 0.01$. No $\delta^{13}C$ value was available for the minke whale. Humpback whales had significantly higher $\delta^{15}N$, but significantly lower $\delta^{13}C$ values. The minke whale was isotopically indistinguishable from humpbacks, but had a significantly higher $\delta^{15}N$ value than finbacks.

Table 4.8. Various statistical tests of isotopic variability between and within prey species: a) geographic variability in capelin for 1994 (S. Todd unpub. data) and 1995/6 (J. Lawson and V. Lesage unpub. data); b) geographic variability in other species (MAB = Mid-Atlantic Bight; data from Montevecchi *et al.* 1992; Abend and Smith 1997); and c) species variability (J. Lawson and V. Lesage unpub. data). Scheffé tests for individual differences between species means are shown in Figure 4.14. K-W = Kruskal-Wallis; M-W = Mann-Whitney.

a)

	1994 ($\bar{x} \pm SD$, ‰)				1995/6, ($\bar{x} \pm SD$, ‰)		
Area	*n*	$\delta^{13}C$	$\delta^{15}N$	CIG	*n*	$\delta^{13}C$	$\delta^{15}N$
GZ1	2	-19.2	13.1 ± 0.05			-	-
GZ2	-	-	-		3	-19.4 ± 0.21	14.4 ±0.64
GZ3	4	-19.0 ±0.19	13.4 ±0.07			-	-
GZ4	7	-18.8 ±0.10	13.1 ±0.20			-	-
GZ5	5	-19.3 ±0.42	13.5 ±0.44		8	-20.3 ± 0.91	13.7 ± 0.65
Test type		K-W	K-W	Wilk's		M-W	M-W
Test statistic		$H = 6.98$	$H = 4.50$	$\lambda = 0.17$		$U = 4.00$	$U = 3.00$
Significance		*ns*	*ns*	*ns*		*ns*	*ns*

ns = not significant, $^{*}p < 0.05$, $^{**}p < 0.025$, $^{***}p < 0.01$.

b)

		herring			mackerel	
		n	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	n	$\delta^{15}N$ (‰)
	GZ1	3	-20.3 ±0.91	13.8 ±0.68		-
	GZ3	-	-	-	4	12.9 ±0.8
	GZ5	26	-20.0 ±0.93	13.4 ±0.53	-	-
	MAB		-	-	9	12.2 ±0.2
Test type			M-W	M-W		t-test
Test statistic			$U = 33.00$	$U = 23.00$		$t = 4.1$
Significance			*ns*	*ns*		***

ns = not significant, $^{*}p < 0.05$, ** $p < 0.025$, $^{***}p < 0.01$.

c)

Species	n	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	CIG
Boreogadus saida	10	-18.8 ±0.29	13.7 ±0.43	
Ammodytes sp.	10	-19.9 ±0.88	12.0 ±0.25	
Pandalus borealis	10	-17.9 ±0.25	11.3 ±0.21	
Gonatus sp.	10	-18.5 ±0.36	12.3 ±0.71	
Mallotus villosus	11	-20.1 ±0.87	13.9 ±0.71	
Clupea harengus	29	-19.9 ±0.88	13.4 ±0.55	
Test type		ANOVA	ANOVA	Wilk's
Test statistic		$F = 22.25$	$F = 43.93$	$\lambda = 0.10$
Significance		***	***	***

ns = not significant, $^{*}p < 0.05$, ** $p < 0.025$, $^{***}p < 0.01$.

were observed between herring from the northeastern coast of Newfoundland and the Gulf of St. Lawrence ($\delta^{13}C$, $p = 0.67$; $\delta^{15}N$, $p = 0.25$; Table 4.8b; J. Lawson and V. Lesage unpub. data). However, over larger geographic distances, differences in isotopic ratios could be detected. For example, mackerel collected from Newfoundland inshore waters differed from mid-Atlantic Bight mackerel ($\delta^{15}N$, $p < 0.011$; Table 4.8b; Montevecchi *et al.* 1992; Abend and Smith 1997).

In addition to geographic differences, temporal changes in prey-isotopic values (Figure 4.13) were examined that suggested yearly isotopic differences in capelin and herring. Non-parametric analysis suggested a relationship between years and $\delta^{15}N$ values for capelin ($\rho = 1.00$, $p = 0.05$). However, no relationship was observed between year and capelin $\delta^{13}C$ values ($\rho = 0.80$, $p = 0.17$) Also, neither herring isotopic variable was correlated with year ($\delta^{15}N$, $\rho = 1.00$, $p = 0.08$; $\delta^{13}C$, $\rho = 0.80$, $p = 0.17$). Qualitatively, isotopic ratios in these prey species have been increasing since 1984/5, but statistics cannot confirm this trend due to the lack of appropriate data (Figure 4.13; J. Lawson and V. Lesage unpub. data; S. Todd unpub.data; Dickson 1987; Hobson and Montevecchi 1991; Ostrom *et al.* 1993; Hobson *et al.* 1996).

Because the above data suggested that prey-isotopic values may vary by time sampled, interspecies variation was examined within one collection season for various locations around Newfoundland and Labrador (J. Lawson and V. Lesage unpub. data; Table 4.8c). Species could be distinguished by CIG, $\delta^{13}C$ and $\delta^{15}N$ levels ($p < 0.01$ for all cases; Table 4.8c; Figure 4.14).

4.3 Correlations between whales and prey

Linear regression exhibited no statistical relationship between isotopic variables (either CIG, $\delta^{15}N$ or $\delta^{13}C$) and DoY, for any combinations of year and location (GZ). It has been previously noted, however, that the relationship between isotopic variable and DoY should not be linear. Thus non-linear trends in $\delta^{15}N$ and $\delta^{13}C$ as function of DoY should also be examined. Levels of $\delta^{15}N$ and $\delta^{13}C$ for humpback whales for each year and GZ are presented in

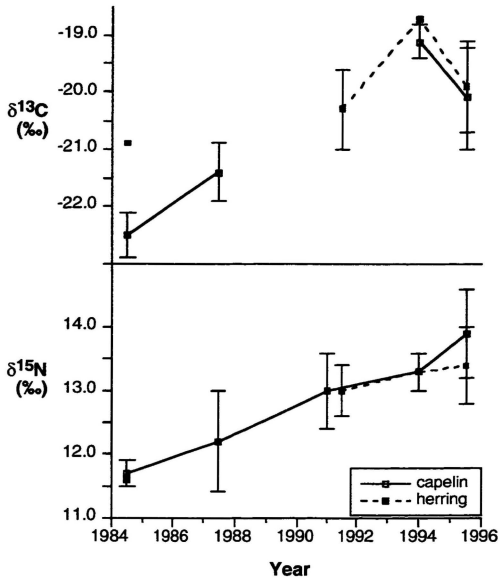


Figure 4.13. Interannual isotopic variation in capelin and herring caught in Northwest Atlantic studies between 1984 and 1996. Data for both species suggest increasing isotopic ratios (both $\delta^{13}C$ and $\delta^{15}N$) since 1984/5.

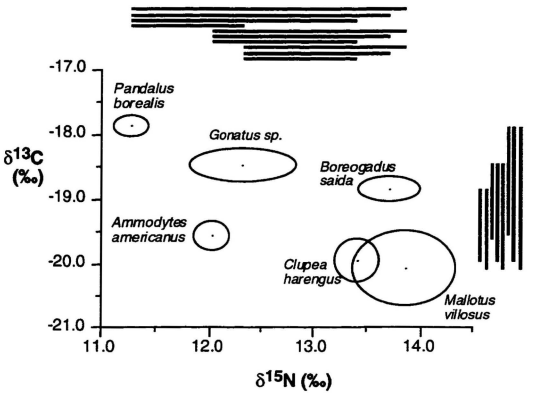


Figure 4.14. Isotopic differences among various prey species sampled in Newfoundland and Labrador waters in 1995/6 (J. Lawson and V. Lesage unpub. data). Bars indicate differences between species means using Scheffé tests at $\alpha = 0.01$ (solid) or $\alpha = 0.05$ (dashed). *Pandalus borealis* was significantly different from most species. Capelin and herring were statistically indistinguishable. Arctic cod had similar $\delta^{15}N$, but dissimilar $\delta^{13}C$ values to capelin and herring.

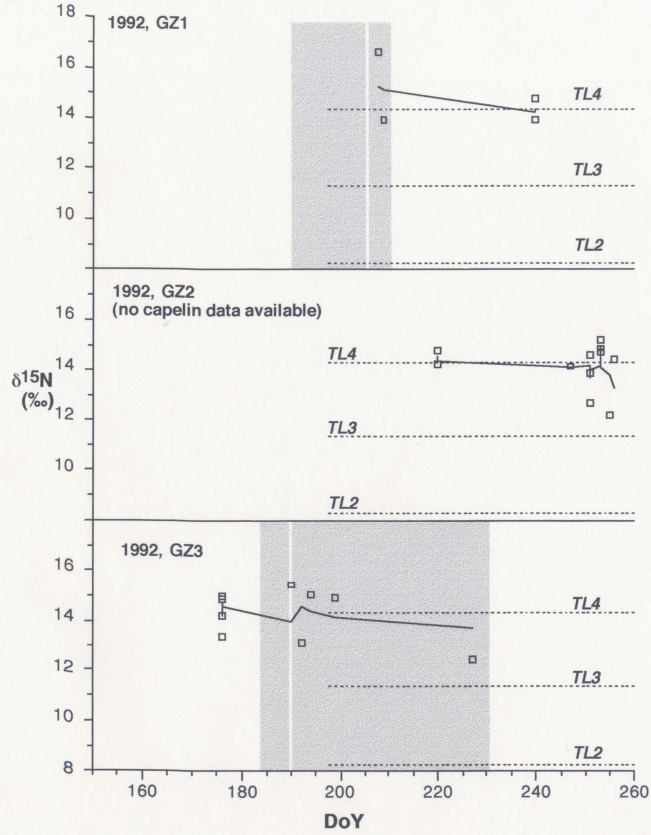
Figures 4.15 and 4.16 respectively (not all combinations of year and GZ are displayed because of low *n*s in some data sets). Trends in isotopic variability due to feeding are best seen in plots of $\delta^{15}N$ versus DoY (Figure 4.15), because of the larger fractionation associated with $\delta^{15}N$ per trophic level. Complete time series were not available due to the opportunistic nature of the sampling. However, smoothing suggest trends that are generalizable to most of the time series. Most humpbacks fed at ~TL4, similar to other piscivores shown in Figure 3.4. A peak in humpback $\delta^{15}N$ values was often observed following the onset of capelin spawning. This delay between the onset of spawning and peak isotopic value ranged between 5–16 days. Humpback $\delta^{15}N$ values tended to decrease after the capelin spawning period. In 1994, the difference between the peak humpback $\delta^{15}N$ value and capelin mean $\delta^{15}N$ value was 2.1‰ for GZ3, and 3.6‰ for GZ4, consistent with a trophic enrichment of 3‰, a value commonly accepted for $\delta^{15}N$ values (DeNiro and Epstein 1981; Minigawa and Wada 1984).

Examinations of plots of $\delta^{13}C$ versus DoY are less clear (Figure 4.16). Trends in $\delta^{13}C$ values are often similar, but less magnified compared to their $\delta^{15}N$ counterparts. It difficult to identify any specific peaks associated with capelin spawning (but see 1993; GZs 4 and 5), or trends following capelin activity. In 1994, when capelin isotopic data were available, the difference between capelin and humpback isotopic values suggest a trophic enrichment for $\delta^{13}C$ ranging from 1.0‰ (GZ3) to 1.3‰ (GZ4), consistent with previous studies (for example, see DeNiro and Epstein 1978).

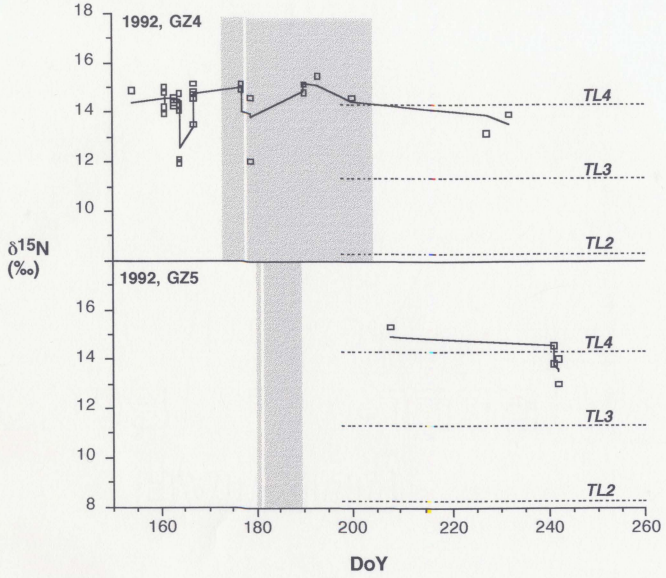
Finback data from 1994 are illustrated and compared with humpback whales sampled in the same time period and GZ in Figure 4.17. The temporal separation seen between the two species in part reflects the earlier arrival of the finbacks in this area. Finbacks clearly have lower $\delta^{15}N$ values compared to humpbacks, implying a lower position in the food web (TL3, although two finbacks have even lower values). The mean finback $\delta^{15}N$ value is also below that of capelin sampled simultaneously in the same area. Finback $\delta^{13}C$ values are indistinguishable from those of humpbacks. Only one minke whale was sampled in this study (1994, DoY = 224).

Figure 4.15. Humpback whale $\delta^{15}N$ values by Day of Year (DoY), for a) 1992, b) 1993, and c) 1994, showing their relationship to availability of capelin. The grey areas cover the spawning period of capelin; the peaks of spawning are indicated by the vertical white lines. Isotopic ratios for capelin in 1994 are available in Table 4.8a. A trophic map is superimposed on each plot based on data from Table 3.6 (TL = trophic level). Humpback whale data were fitted with a running average smoothing algorithm (solid line; Chapter 3).

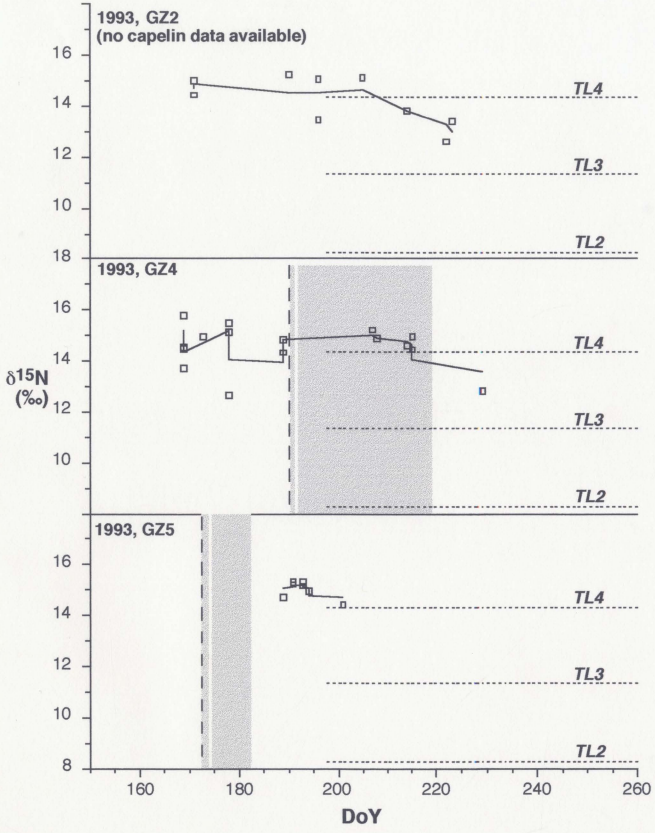
a)


1992, GZ1
TL4
TL3
TL2
1992, GZ2
(no capelin data available)
TL4
TL3
TL2
1992, GZ3
TL4
TL3
TL2
δ15N
(‰)
18
16
14
12
10
16
14
12
10
16
14
12
10
8
160
180
200
220
240
260
DoY

a)continued...


1992, GZ4
1992, GZ5
TL4
TL3
TL2
δ15N
(‰)
DoY
18
16
14
12
10
8
160
180
200
220
240
260

b)


1993, GZ2
(no capelin data available)
TL4
TL3
TL2
1993, GZ4
TL4
TL3
TL2
1993, GZ5
TL4
TL3
TL2
δ15N
(‰)
16
14
12
10
18
16
14
12
10
16
14
12
10
8
160
180
200
220
240
260
DoY

c)

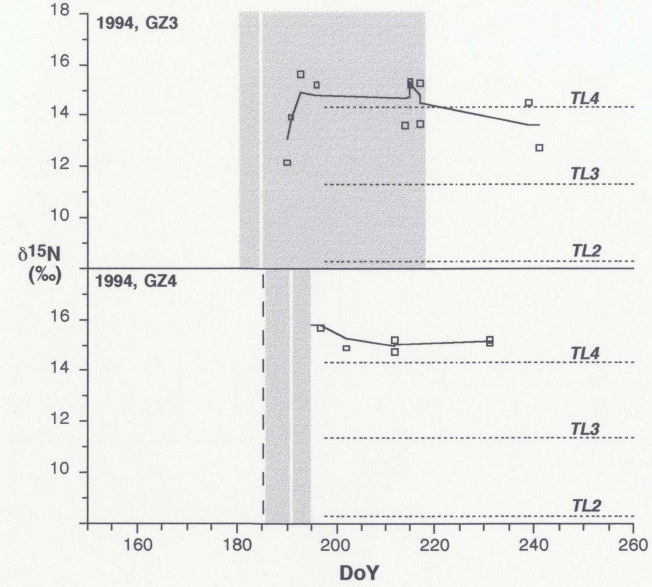
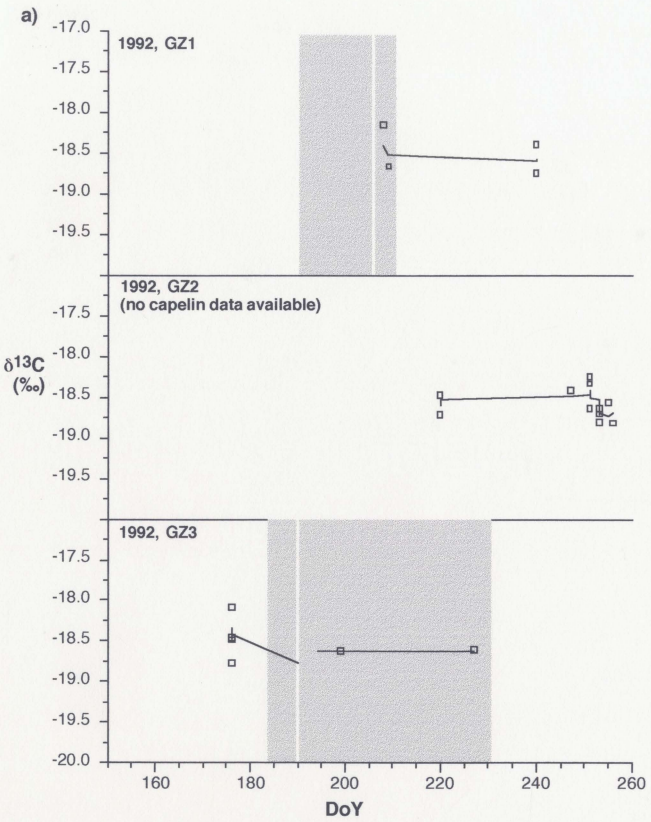
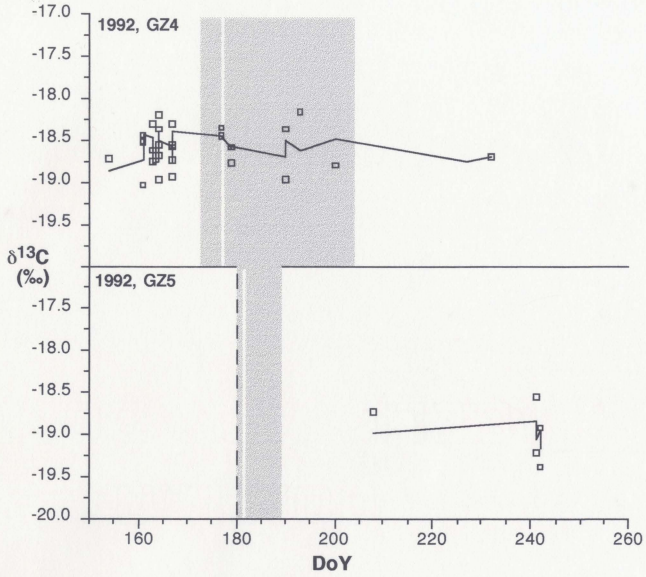
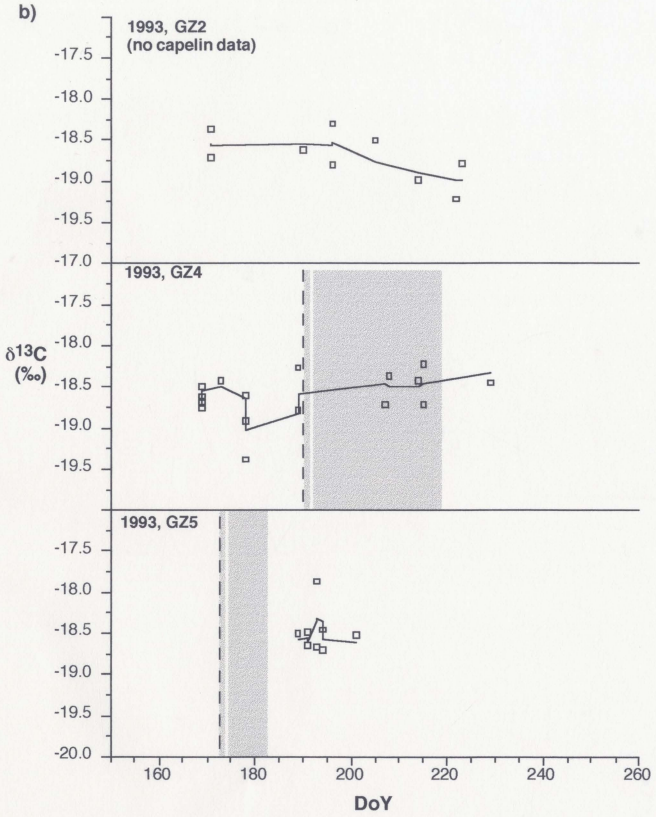

1994, GZ3
1994, GZ4
TL4
TL3
TL2
δ15N (‰)
DoY
18
16
14
12
10
160
180
200
220
240
260

Figure 4.16. Humpback whale $\delta^{13}C$ values by Day of Year (DoY), for a) 1992, b) 1993, and c) 1994, showing their relationship to availability of capelin. The grey areas cover the spawning period of capelin; the peaks of spawning are indicated by the vertical white lines. Isotopic ratios for capelin in 1994 are available in Table 4.8a. Humpback whale data were fitted with a running average smoothing algorithm (solid line; Chapter 3).

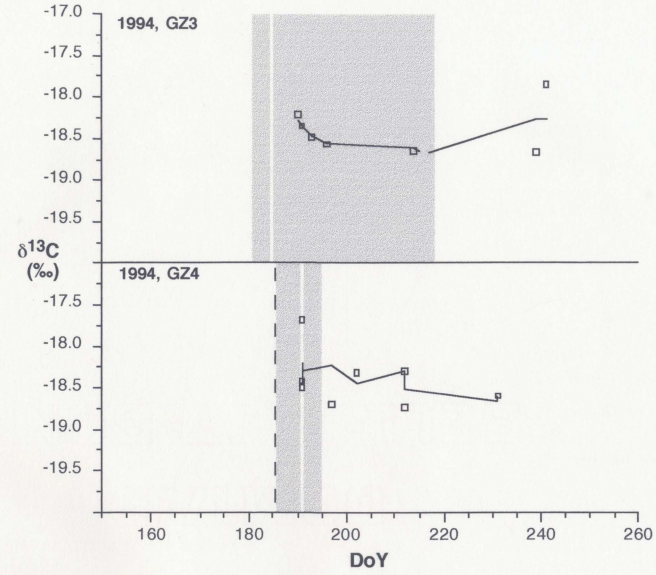

a)
1992, GZ1
1992, GZ2
(no capelin data available)
1992, GZ3
δ13C
(‰)
-17.0
-17.5
-18.0
-18.5
-19.0
-19.5
-20.0
160
180
200
220
240
260
DoY

a) continued...


1992, GZ4
1992, GZ5
δ13C
(‰)
-17.0
-17.5
-18.0
-18.5
-19.0
-19.5
-20.0
160
180
200
220
240
260
DoY


b)
1993, GZ2
(no capelin data)
1993, GZ4
1993, GZ5
δ13C
(‰)
-17.5
-18.0
-18.5
-19.0
-19.5
-17.0
-20.0
160
180
200
220
240
260
DoY

c)


1994, GZ3
1994, GZ4
-17.0
-17.5
-18.0
-18.5
-19.0
-19.5
δ13C
(‰)
160
180
200
220
240
260
DoY

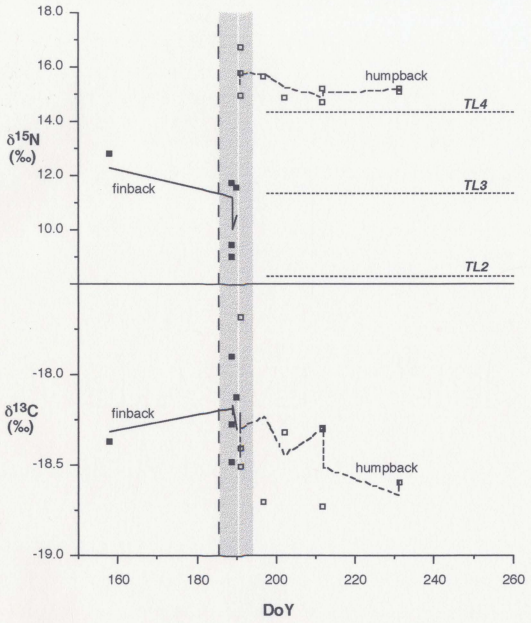


Figure 4.17. Humpback and finback whale $\delta^{15}N$ and $\delta^{13}C$ values sampled in GZ4, 1994. Capelin date of spawning range is delimited by the grey block, with peak spawning denoted by a vertical white line. A dashed line to the left of the grey block indicates uncertainty concerning the start of the spawning period. Trophic levels (TL) based on data from Table 3.6 are also plotted. Whale data have been fitted with a running average smoothing function (Section 3.11).

However, the minke whale's $\delta^{15}N$ value of 15.5‰ is consistent with humpbacks biopsied at similar times in the same area (see Figure 4.15c, GZ3), suggesting the animal was feeding at TL4, potentially on the same prey species.

5. Discussion

5.1 Can SIA be used in studies of cetacean foraging ecology using biopsy material?

Biopsy samples taken from free-ranging cetaceans can be used as source tissue for SIA (Chapter 2; Todd *et al.* in press). In this section, four important considerations of SIA are discussed in reference to findings in Chapter 2—specifically: the turnover time of the tissue being analyzed; the nutritional state of the animal being measured; homogeneity of isotopic signal within the sampled tissue and within the animal; and the values for f_N and f_C, the isotopic fractionation associated with trophic enrichment.

5.1.1 Rate of turnover in cetacean tissues

The isotopic similarity reported in Chapter 2 between muscle and skin tissue taken from humpbacks has several possible explanations. For example, the turnover rates for muscle and skin tissue may be relatively similar, and thus these tissues might reflect diets taken in similar time periods. Metabolic evidence suggests that, of the two tissues, skin tissue likely has a briefer turnover period, at least in non-fasting animals such as those sampled in this thesis (Altman and Dittmer 1968; Waterlow *et al.* 1978; Lobley *et al.* 1980; Attaix *et al.* 1988; Lobley *et al.* 1992). Blubber tissue likely has a long metabolic turnover. In fasting animals, this relationship may change with the defense of protein reserves and the mobilization of body fats (Castellini and Rea 1992; Markussen *et al.* 1992b).

Empirical data for rate of tissue turnover in cetaceans are not available. It is often assumed that muscle tissue has a metabolic half-life of ~27 days, based on estimates from vertebrate

species other than marine mammals (Tieszen *et al.* 1983; Ostrom *et al.* 1993; Hobson *et al.* 1996). Hobson and Clark (1992a) found a higher metabolic turnover in quail pectoral muscle (~12 days), although it is possible that this is explained by the highly active nature of flight, and quail's higher resting-metabolic rate. Turnover rates in skin and muscle may not be similar; for example, differences in the rate of protein synthesis in muscle and skin tissue suggest that skin is capable of more rapid turnover (Waterlow *et al.* 1978; Lobley *et al.* 1980; Attaix *et al.* 1988). Thus, if the metabolic half-life of muscle tissue is estimated at ~20 days, one may assume that the half-life of skin tissue is less than this value. Anecdotal evidence presented in this thesis suggests the turnover rate for skin in humpback whales is ~7–14 days (Sections 4.3 and 5.2.3).

If muscle and skin have different turnover rates, isotopic similarity between the two tissues may be a function of animals feeding on diets that were homogenous over the integration periods of either tissue. From the above assumptions, for muscle and skin to be isotopically similar due to a homogenous diet, the diet of the animal would need to be isotopically constant for greater than 20 days, with similarity between the two tissues with respect to $\delta^{13}C$ and $\delta^{15}N$ improving further beyond that time. A majority of the animals in the pilot study were sampled in mid-July or later, when evidence suggests diet may vary (Section 4.1.3; 5.2.3). Therefore, it seems unlikely that isotopic similarities between skin and muscle tissue are due to consistencies in diet.

A third explanation of the isotopic similarity between muscle and skin tissue is that lack of recent feeding may have caused fasting. In such instances, mobilization of protein and other reserves may have equalized isotopic composition in both tissues (aspects of nutritional stress and fasting are discussed in Section 5.1.2).

5.1.2 Fasting and nutritional stress

Isotopic similarity between muscle and skin tissue may have been caused by recent 'forced' fasting by the animals. All sampled whales reported in Chapter 2 were either stranded or killed in fishing gear. The lack of a systematic sampling schedule for strandings would likely bias the type of animals selected (J. Lien pers. comm.). In addition, the causes of both stranding and net-entrapments remain unknown (Klinowska 1986; Brabyn and McLean 1992; Geraci and Lounsbury 1993; Perrin *et al.* 1994), but one possibility is that animals make navigational errors (Lien 1994). Lack of sufficient orientation skills might also debilitate feeding efforts, causing an unnatural fast. Thus stranded animals may not provide representative samples from animals consuming a normal diet.

Furthermore, fasting animals may have uncharacteristic isotopic values (Gannes *et al.* 1997). For example, Hobson *et al.* (1993) suggest that isotopic ratios become enriched with respect to the trace isotope when an animal is metabolically stressed. Fasting may also result in mobilization of food reserves that may influence a consumer's isotopic composition. Polar bears demonstrate little variability in isotopic composition in periods of reduced feeding, despite reliance on isotopically different diets. This might be due to a greater reliance on fat reserves at such times (Hobson and Stirling 1997).

Unfortunately, no comparative data are available to suggest uncharacteristic, fasting-induced isotopic levels for the humpbacks reported in Chapter 2. Clearly, the use of stranded animals has the potential to create biases in studies of diet by either analysis of gut-contents or SIA. More data are needed to demonstrate isotopic changes in fasting animals.

5.1.3 Signal homogeneity within tissues

A third assumption of SIA is that a sample provides a representative isotopic value of the tissue it comes from. That is, it is assumed that signal homogeneity exists within tissues. A majority of previous isotopic studies examined animals in which large percentages of particular tissues, or even the entire organism, could be sampled. A biopsy of a free-ranging whale, however, samples only a minute proportion of the overall tissue. Furthermore, while a carefully fired dart can guarantee a core of tissue that samples the entire depth of skin, only the top layer of blubber is sampled—which is thought to be biochemically different to other blubber layers (Lockyer *et al.* 1984; Lockyer 1987). Thus, blubber may not be isotopically homogenous with respect to layer depth.

Schell *et al.* (1989a) present the only data which investigate the issue of homogeneity of isotopic signal between different locations of the same tissue in cetaceans, but these comparisons are limited to metabolically inert baleen. No data are available on isotopic variation within cetacean skin or blubber cores, or between cores taken from different locations on the animal. For example, some biopsies in this study took skin tissue from the ventral surface of the tail, which has a visibly different consistency to that of skin taken from the dorsal surface of the animal. Differences in location of biopsy were not recorded by YoNAH protocol; thus if isotopic differences exist between biopsy locations, this would be an unquantifiable source of error to the data set presented here.

Data presented in Chapter 4 (for example, Figure 4.1) suggest that overall, isotopic composition can be highly variable. Excluding outliers, the range for humpback $\delta^{15}N$ values was 4.8‰, and for $\delta^{13}C$ was 1.7‰. While some of this variability may be due to individual differences in diet, it is also possible that a high degree of variability exists within any one tissue of the animal.

In the absence of data that demonstrate isotopic homogeneity within tissues of the same individual, a first approximation of the effects of sampling error can be obtained by examining data from individuals that were resampled within a brief period of time. Such data exist for eight individuals resampled in 1992. In Figure 4.8b, magnitude of isotopic change is plotted against length of sampling interval. When feeding on isotopically-unique dietary items that display distinct temporal availability, isotopic composition of the consumer will change with time. In Figure 4.8b, it is expected that isotopic change will be small for animals with brief resampling intervals. Correspondingly, larger isotopic differences are expected for animals with longer resampling periods. Thus, in general, Figure 4.8b should demonstrate a positive relationship between magnitude of isotopic change and length of resampling interval, for either isotopic variable. Sampling error will tend to obscure this pattern, because each isotopic value will be the sum of: a) treatment effects, and b) sampling error.

With respect to $\delta^{15}N$ values, the high value of r^2 (0.88) in Figure 4.8b indicates sampling error is low. However, $\delta^{13}C$ effects seem more susceptible to sampling error ($r^2 = 0.26$), likely because fractionation effects associated with $\delta^{13}C$ values per trophic level are smaller than for $\delta^{15}N$ values. These data further support an emphasis on $\delta^{15}N$ values as an appropriate indication of trophic change. Thus the data suggest that sampling error is minimal within skin tissue, certainly with respect to $\delta^{15}N$ values. This thesis assumes that isotopic homogeneity exists within a tissue, although further data are needed to conclusively prove this.

Two biopsy samples in this study came from unusual skin sources: skin from the 1995 ice-entrapped humpback whale came from tissue scraped off the flukes by encroaching pan-ice; and tissue from the 1994 minke whale came from skin sloughed by the animal from the tail. Isotopic values associated with sloughed, dead skin may be unusual. The ice-entrapped humpback has an anomalously low $\delta^{15}N$ isotopic value in comparison to other humpbacks sampled in 1992-1994, but whether this was due to some aspect of the animal's health, feeding on lower trophic

level prey (this animal was sampled very early in the year), fasting, or some unique biochemical aspect of skin taken from the tail, is not known.

Few comparative data are available for minke whales: previous estimates from dead animals include Ostrom *et al.* (1993; $\delta^{15}N$ = 12.3‰, n = 1) and P. Ostrom and J. Lien (unpub. data; $\delta^{15}N$ = 13.2 ± 1.96‰, n = 3). Such values are substantially lower than the living animal measured in this study. Similar to the above, it is not known if these differences are the result of some distinction of internal state within the animals measured, or because of a unique biochemistry of dead, sloughed tissue. Ames *et al.* (1996) demonstrated that sloughed skin taken from live captive manatees had lower $\delta^{13}C$ values in comparison to skin sampled from dead, wild manatees. Although Ames *et al.* attribute this to differences in diet, a second explanation might be a fractionation occurring in dying or decaying tissue, resulting in enriched levels of the trace isotope. The use of sloughed skin as a tissue source for SIA has an appealing non-invasive aspect, but more data are required on the biochemical nature of such tissue.

5.1.4 Use of f_N and f_C as fractionation constants associated with trophic enrichment

A third assumption in the use of SIA in ecological studies are the values of f_N and f_C, the fractionation constants associated with trophic enrichment (Equation 2, Chapter 1). Evidence strongly suggests that f_N = ~3‰ and f_C = ~1‰ (DeNiro and Epstein 1978, 1981; Minigawa and Wada 1984). However, Gannes *et al.* (1997) suggest that f_N and f_C may not be as constant as previously believed. It is known that fractionation differs across tissues (Tieszen *et al.* 1983; Hobson and Clark 1992b; Hobson *et al.* 1996), and as a function of metabolic stress (Hobson *et al.* 1993; Gannes *et al.* 1997). Abend and Smith (1995; 1997) suggest that $\delta^{15}N$ trophic fractionation may be less than 3‰ in cetaceans. The assumption of values of f_N and f_C are further discussed below for particular sources of isotopic variation.

5.2 What range of isotopic values typify the Northwest Atlantic population of humpback whales?

Humpback $\delta^{15}N$ values are clearly more variable than their corresponding $\delta^{13}C$ measurements. This can be expected, given that $\delta^{15}N$ levels are more sensitive to changes in diet (DeNiro and Epstein 1978, 1981; Minigawa and Wada 1984). The high variability in either variable may be a function of: a) measurement precision (within ±0.2‰ for this study); b) sampling error (heterogenity of signal within a tissue); c) differences in diet history; or d) internal factors (size, health, etc.; see Gearing 1991).

Isotopic values obtained in this study for humpback whales are in general agreement with the few data collected to date. For example, the average $\delta^{15}N$ (pooled across all treatment variables) measured in this study of 14.6 ± 0.94‰ ($\bar{X} \pm SD$) is similar to published (13.4‰, n = 1; Ostrom *et al.* 1993) and unpublished ($\delta^{15}N$ = 13.6 ± 0.89‰, n = 15; P. Ostrom and J. Lien unpub. data) values of humpback muscle sampled prior to 1992. The mean $\delta^{13}C$ value of -18.5 ± 0.28‰ is also similar to the non-lipid-extracted muscle value of -18.7‰ (n = 1) obtained by Ostrom *et al.* (1993), although the low n of the latter study precludes meaningful comparisons for either variable. However, the $\delta^{13}C$ value recorded here is low compared with lipid-extracted values of muscle (e.g., $\delta^{13}C$ = -17.8 ± 0.4‰, n = 11; P. Ostrom and J. Lien unpub. data). Such comparisons should be made with caution as both previous assessments came from stranded animals prior to 1991, and are thus subject to biases discussed in Section 5.1.2, as well as other unmeasured ecological factors. Furthermore, as discussed in Section 5.2.4, evidence presented in this thesis suggests that $\delta^{13}C$ and $\delta^{15}N$ of humpbacks may be changing with time, and therefore comparisons between years should be made carefully.

If the above isotopic values are typical for a feeding substock of humpback whales, one should examine possible causes of the two outliers observed in this study (Section 3.8). Humpback #EC92047, sampled in 1992, had a similar $\delta^{15}N$ but a ^{13}C-enriched isotopic ratio in

comparison to neighbouring values. Two quantifiable differences between this sample and other humpbacks were: the animal was a female; and it had the lowest skin lipid content of any humpback measured in this study (approximately half that of other animals). Low levels of lipids in peripheral tissues are known to occur in lactating females as fats are mobilized for milk production (Lockyer 1986, 1987; Vikingsson 1990); for finback muscle tissue, nursing females have approximately half the amount of lipids seen in anoestrous females (Lockyer 1987). Thus, the anomalous $\delta^{13}C$ value may have been because the animal was lactating (since milk production might discriminate against ^{13}C; see Boutton *et al.* 1988). This hypothesis is partially supported by #EC92047's $\delta^{15}N$ level which suggested that the animal was not feeding on a unique food source. Unfortunately, the hypothesis that #EC92047 was lactating remains as speculation owing to a lack of corroborating evidence; although this animal was part of a group of 3, the remaining 2 animals (one of which should have been #EC92047's calf) were not identified.

Humpback #EC94NF118 was also considered an outlier, although only one isotopic measurement ($\delta^{15}N$) was available. This animal's skin had a high lipid content (17.4%), although not statistically different from the remaining humpbacks. If this outlier was not due to experimental error, this animal may have recently fed on prey different to that taken by the majority of humpbacks sampled in this study.

5.2.1 Sexual differences

Sexual variation in isotopic composition was not observed, suggesting that males and females feed on similar prey. In part, this hypothesis is supported by: a) previous analyses of gut contents which did not demonstrate any differences in diet due to sex (Nemoto 1970; Mitchell 1974; Kawamura 1980); and b) a lack of dimorphism between sexes in this species (Winn and Reichley 1985).

Anecdotal evidence presented above suggests that lactating females may be ^{12}C-depleted due to mobilization of lipids in milk synthesis. If this is the case, then one might expect to observe a subgroup of females demonstrating higher $\delta^{13}C$ isotopic ratios. Mitchell (1973) reported that 20% of females sampled in a scientific take in 1971 were lactating. Such a proportion was not seen in this study: only 3 of the 58 female humpbacks demonstrated higher $\delta^{13}C$ levels (EC930295, EC94NF151 and EC93121), and only one of these was measured for lipids (EC93121; lipid % by mass = 10.65%). It is possible that YoNAH protocols selected against sampling mother-calf pairs, for several reasons. First, at least on their breeding grounds, mother-calf pairs occupy different areas to the main population, possibly to avoid predators (Smultea 1994). Second, increased vigilance by a mother-calf pair may have made the animals more difficult to approach and thus biopsy. Third, mother-calf pairs tend to migrate later than the rest of the population (Mackintosh 1965; Dawbin 1966), and thus may not be associated with the main grouping of animals that YoNAH protocols prioritized.

5.2.2 Geographic variation

Geographic variation by GZ could not be detected in $\delta^{13}C$ or $\delta^{15}N$ levels. However, when the variables were considered together as a CIG, the different geographic zones could be distinguished. In general, the largest difference between CIG means occurred between areas that were separated by the greatest geographical distance. Specifically, greatest mean difference between geographic zones with respect to $\delta^{13}C$ was between the North and South coasts. A large difference in mean $\delta^{13}C$ levels was also seen between the North Coast and the West Coast/Labrador (although all biopsies in GZ2 came exclusively from the Labrador coast, due to whale distribution). Prey did not differ isotopically by geographic region unless distances between samples was large (Table 4.8a, b), although differences in collection protocol

may confound this finding. When collection procedures were standardized, various species of prey could be distinguished isotopically (Table 4.8c, Figure 4.14).

Carbon isotopic values are typically used to distinguish sources of carbon uptake. This is possible if carbon sources have different $\delta^{13}C$ levels; for example, in comparing C_3 and C_4 plants (Smith and Epstein 1971), types of macroalgae (Stephenson *et al.* 1986; Ostrom and Fry 1993), or terrestrial and marine organic matter (e.g., Chisolm *et al.* 1982; Hobson *et al.* 1997). However, $\delta^{13}C$ levels may vary as a function of other factors. For example, $\delta^{13}C$ levels may be a function of water mass or a particular species composition of phytoplankton (Rau *et al.* 1982; Fry *et al.* 1984; Saupe *et al.* 1989; Fogel and Cifuentes 1993). It is frequently observed that $\delta^{13}C$ levels measured in photosynthesized organics of primary producers are inversely proportional to aqueous concentrations of CO_2, which are in turn inversely related to rates of primary production (Rau *et al.* 1989; Fogel *et al.* 1992; Fogel and Cifuentes 1993; Goericke *et al.* 1994). Thus, $\delta^{13}C$ values may indicate levels of primary production, although it is recognized that this relationship also depends upon phytoplankton growth-rate and species composition (Laws *et al.* 1995). Upwellings may recycle ^{13}C depleted carbon, creating unique geographic gradients in $\delta^{13}C$ (Saupe *et al.* 1989). Finally, latitudinal gradients may occur because of temperature changes that affect the solubility of CO_2, causing ^{13}C depleted values in polar regions (Rau *et al.* 1982, 1989; Parsons 1995).

Nitrogen isotopic values vary mainly as a function of trophic level, as well as nutrient availability and species composition (DeNiro and Epstein 1981; Minigawa and Wada 1984; Peterson and Fry 1987). However, Owen (1987) noted that $\delta^{15}N$ levels change with depth due to decomposition of organic materials.

Geographic variation in $\delta^{13}C$ or $\delta^{15}N$ levels has been used in several SIA studies of cetaceans. Schell *et al.* (1989a, 1989b) and Schell and Saupe (1993) use recorded geographic $\delta^{13}C$ variations in plankton (Saupe *et al.* 1989) to demonstrate the dependence of bowhead whale on zooplankton in either the Bering, Chukchi or Beaufort Seas. Abend and Smith (1995;

1997) used differences in $\delta^{13}C$ and $\delta^{15}N$ values in skin, muscle, blubber and teeth of North Atlantic long-finned pilot whales (*Globicephala melas*) to distinguish between eastern and western populations.

In this study, it was noted that isotopic differences between humpbacks in each GZ, even when statistically significant, tended to be small, potentially within measurement error of the analysis equipmet (±0.2‰; Chapter 2). If not a measurement artifact, isotopic differences between geographic zones may partially be a consequence of variability in plankton composition (Dickson 1987; Anderson and Dalley 1996). Differences in water mass may also be important, given the proportional contribution of the Gulf of St. Lawrence and the Labrador Current within each GZ (Myers and Akenhead 1988; Drinkwater and Trites 1993; Drinkwater *et al.* 1994). The western and southern coasts of Newfoundland (GZ2, 5) are influenced by the Gulf of St. Lawrence (Colbourne 1994), whereas the eastern coast (GZ 1, 3, 4) is more affected by the Labrador Current. Differences between GZs due to oceanographic influences can be seen in $\delta^{13}C$ values (Figure 4.3); for example, GZ4 (which incorporates some southern coasts) has an intermediate $\delta^{13}C$ values between two distinct groups; northeastern (GZs 1, 3) and southern/western areas (GZs 2, 5). These differences are confirmed when GZs are statistically grouped on this basis and compared to each other (Table 4.2b).

Depletion of ^{15}N with latitude seen in this study may have been a statistical artifact due to an uneven spread of values with latitude: alternatively, the data may be confounded temporally. However, it is also possible that the observed depletion was a function of variation in prey availability at different latitudes; specifically, lower trophic-level feeding in more northern areas. For example, Lear (1979) and Lilly *et al.* (1994) document the distribution of Arctic cod as being more abundant in northern areas of Newfoundland. Balaenopterids have been anecdotally reported as feeding on Arctic cod in White Bay (GZ1; J. Lien pers. comm.). Arctic cod may have different $\delta^{15}N$ levels to that of other humpback whale prey, although preliminary data from 1996 suggests that Arctic cod did not have a lower value

of $\delta^{15}N$ than capelin taken in the same year (J. Lawson and V. Lesage unpub. data; Table 3.5; Figure 3.4). More data on required on the isotopic compositions of locally available prey-items sampled at the same time as the consumer.

5.2.3 Seasonal variation

Intra-annual isotopic variability has been used in cetacean studies to demonstrate seasonal feeding on isotopically unique prey sources. For example, Schell *et al.* (1989a, 1989b), and Schell and Saupe (1993) demonstrated seasonal isotopic changes in baleen plate strata, which indicated differences in diet within an annual migration. Hobson *et al.* (1996) performed a similar analysis based on the analysis of isotopic values along the length of whiskers taken from captive seal. Abend and Smith (1995; 1997) suggested differences in isotopic composition between muscle and skin were a function of the turnover rate of each tissue; therefore, signals in either tissue corresponded to feeding at different times within the year.

Two marine-mammal studies examined longitudinal changes in the isotopic composition of same-individuals, based on repeated non-invasive sampling techniques. Ames *et al.* (1996), using sloughed skin, demonstrated changes in consumer $\delta^{13}C$ that were related to diet. Hobson and Sterling (1997) isotopically analyzed polar bear blood samples and showed nutritional dependence on either blubber reserves or supplemental feeding during periods of low food availability.

In this study, considerable intra-annual variation occurred in humpback isotopic CIGs and values per year, beyond that accountable to errors in precision. On a month-to-month basis, a cyclical pattern was observed yearly, with low isotopic values in June rising to a peak in July, followed by a drop in August and September. However, this effect was only large enough to be statistically significant with respect to $\delta^{15}N$ values. When intra-annual variation was expressed as Day of Year (DoY), $\delta^{13}C$ and $\delta^{15}N$ values decreased throughout the year,

reflecting the drop in values between July and August (although none of the correlations between DoY and isotopic variable for any year were significant).

Several other methods were used to demonstrate the seasonal trends in $\delta^{13}C$ and $\delta^{15}N$. First, changes in isotopic values in repeatedly sampled individuals were examined (Section 4.1.4; Figure 4.8). Isotopic composition changed in most individuals; it is not known how much of this variation was due to sampling error or treatment effects (although by inference from Section 5.1.3, sampling error is likely minimal). Generally, $\delta^{15}N$ values changed more than $\delta^{13}C$ values, a reflection of the greater sensitivity of $\delta^{15}N$ levels to dietary change. Since the resampling occurred early in the feeding season (DoY ~ 152–168) it is likely that these animals had just begun feeding in Newfoundland waters.

Second, time-series data for all combinations of year and GZ were presented (Section 4.3; Figures 4.15, 4.16). It should be reiterated that these series do not correspond to longitudinal sampling of the same individual. Rather, they referred to sequential sampling of a group of whales in the same geographic area in the same year. Presumably, within such groups, prey availability would be relatively consistent. Thus time-series data from a group of animals might result in trends that would be similar to those seen in a longitudinal sampling program of the same individual. Unquantified individual differences such as arrival date, feeding history, as well as initial isotopic compositions prior to feeding, as dictated by the physiology and health of the animal, would also influence such trends (Gearing 1991; Hobson *et al.* 1993; Gannes *et al.* 1997).

For each combination of year and GZ, $\delta^{15}N$ values varied more than $\delta^{13}C$ values. This was expected, since ^{15}N has an associated trophic enrichment approximately three times greater than ^{13}C. In many cases, peaks in $\delta^{15}N$ were seen in late July, usually following the onset in capelin spawning by 7–17 days ($\bar{x} \pm SD = 13 \pm 5.0$ days, $n = 3$) and the peak in capelin spawning by ~5–16 days (12 ± 4.8 days, $n = 5$). In 1994, when capelin isotopic data were available by GZ, humpback peak $\delta^{15}N$ values were ~2.5–3‰ above that of the mean value for capelin for that

GZ. Corresponding $\delta^{13}C$ time series suggested humpback isotopic ratios ~1‰ above those of capelin for that GZ. These data imply that the isotopic peak seen in humpbacks in July corresponds to the seasonal availability of capelin, and reaffirm the use of 3‰ and 1‰ as fractionation constants for f_N and f_C respectively. Furthermore, the delay between capelin spawning and peak humpback $\delta^{15}N$ values represents a first approximation of the integration time of humpback skin tissue, ~1–2 weeks.

The time series of isotopic values presented in Figures 4.15 and 4.16 also demonstrated other trends. Some of the variability in isotopic composition seen here was due to individual differences, either in physiological state, size, or in recent feeding history (humpbacks may begin their feeding during their migration from the southern breeding grounds). However, it is possible that other trends and peaks represented recent feeding on other, isotopically distinguishable prey species. For example, examination of monthly data suggested that $\delta^{15}N$ values may have increased in September, although this is based on data from one year only. Prey available later in the feeding season include Arctic cod and various species of squid.

If yearly data are pooled, the same monthly variations in humpback $\delta^{13}C$ and $\delta^{15}N$ ratios are observable. CIGs derived from pooled data suggest good separation by month, although this was primarily caused by differences in $\delta^{15}N$, not $\delta^{13}C$. Although $\delta^{13}C$ values did not vary significantly by month, they showed the same trends seen in $\delta^{15}N$ (Figure 4.6).

Section 3.7 discusses methods that allow the pooling of data across years. One possible mechanism, that of the use of a coarse time scale such as months, works effectively to remove slight differences in synchronization between years (see Figure 4.5b). Use of common biological events such as capelin spawning to synchronize data is an alternative method; no difference in prediction was seen between either method of synchronization (i.e., by peak spawning or by first onset of spawning of capelin). Also, neither synchronization method offered significant improvements in prediction of $\delta^{15}N$, $\delta^{13}C$ or CIGs, suggesting years were already closely synchronized prior to adjustment. All assessments of isotopic variables plotted against pooled

DoY yielded negative relationships, a function of the isotopic peaks seen earlier in the season (July), although the statistical significance of these analyses might be an artifact of the high n used in these particular tests.

5.2.4 Interannual variation

Variation between years in isotopic composition may be the result of three factors: ontogenetic changes within the consumer that prompt different diets or different biochemical pathways in assimilation (Schell *et al.* 1989a; Ames *et al.* 1996); changes in prey availability (Wainwright *et al.* 1993; Thompson *et al.* 1995); and isotopic changes at the base of the food web prompted by oceanographic factors (Schell *et al.* 1989a; Wainwright *et al.* 1993).

Ontogenetic changes in the consumer may change the efficiency at which certain prey may be hunted, or change the types of prey selected by the consumer. Such changes have been observed isotopically in young bowhead whales by examination of layers of baleen plates that provide yearly accounts of diet (Schell *et al.* 1989). Ames *et al.* (1996) also showed isotopically a young manatee's dependence on maternal milk; subsequently, the isotopic record demonstrated that the manatee switched from a milk-based diet to available vegetation.

Interannual variation in isotopic composition of the consumer may be caused by changes in availability of prey species. For example, Thompson *et al.* (1995) noted a decrease in $\delta^{15}N$ between 1900s and 1990 in northern fulmars (*Fulmarus glacialis*). Thompson *et al.* accounted for this difference by suggesting that the fulmars had switched their foraging strategy from scavenging on offal discarded by the whaling industry to hunting for prey species lower in the food web.

Finally, interannual variation in isotopic composition may be caused by long-term oceanographic factors. For example, Wainwright *et al.* (1993) found $\delta^{15}N$ values of certain fish species were strongly influenced by large-scale oceanographic variables such as the NAO, as

well as partially anthropogenically controlled variables such as species stock size. Two possible isotopic-influencing mechanisms were thus proposed. First, various factors such as changes in water mass characteristics, and the availability of dissolved inorganic carbon and nitrogen, resulted in changes in isotopic composition of phytoplankton. These isotopic anomalies then persisted throughout the food web. Second, changes in isotopic composition may have been caused by variation in prey availability, which would have affected proportions of diet seen in the consumer (Wainwright *et al.* 1993).

Substantial interannual isotopic variation was recorded in this study. This is demonstrated in plots of CIGs and individual isotopic variables by year (Figure 4.10). Over the 6 years sampled, $\delta^{15}N$ levels increased, while $\delta^{13}C$ levels decreased. *Post hoc* testing suggests that the major decrease in $\delta^{13}C$ levels coincided with the major increase in $\delta^{15}N$ levels between 1990–1992. Since this same period represents a change in sampling procedure (switching from dead strandings to live biopsies), differences in isotopic levels due to collection methods should first be addressed. It is concluded here that long-term annual trends were not an artifact of collection procedure, for two reasons. First, it has been argued above that stranded animals should have elevated isotopic ratios (Section 5.1.2). If this is the case, then both $\delta^{13}C$ and $\delta^{15}N$ values sampled from stranded individuals in the time period 1988–1992 should be higher than those seen in 1992–1994. This clearly does not occur (Figure 4.10b). Second, the trend set in isotopic values between 1990–1992 is continued in 1992–1994; such continuity would likely not be observed if collection procedures had an effect on isotopic values. Thus, it is necessary to examine other possible causes of the shifts in isotopic composition seen between 1988–1994. To do this, previous oceanographic and biological data for Newfoundland and Labrador were reviewed.

In marine environments, oceanographic and biological processes are inevitably linked (for example, see Beamish 1995). With reference to the Northwest Atlantic, Mann (1993) and Mann and Drinkwater (1994) document the potential influence of the NAO on biological productivity.

In brief, the NAO is an oscillation of atmospheric pressure differences between the cold air temperature, low pressure system of the Arctic, and the warm air temperature, high pressure system of equatorial regions. Net movement of air towards the polar regions is deflected by the coriolis effect to create dominant geostrophic northwesterly winds in the Northwest Atlantic (Mann and Drinkwater 1994; Morton 1998).

A negative NAO index implies weaker pressure differences between the Pole and Equator, and thus weaker winds. Associated with this are warmer temperatures, shorter periods and less extensive ice cover, and more saline waters. A positive NAO index implies the opposite conditions, with strong northwesterly winds pushing cold Arctic air into the Northwest Atlantic (Myers and Akenhead 1988). The NAO index has been positive since 1980, and termed 'anomalously high' since the late 1980s (Mann 1993, p.19). Newfoundland waters have experienced extended ice covers, low sea surface temperatures and low salinities since the 1990s (Drinkwater and Trites 1993; Colbourne 1994; Drinkwater *et al.* 1994; Colbourne 1995).

Abnormally low salinities, low temperatures and heavy ice cover may have several direct and indirect effects on isotopic ratios in high consumers. First, low temperatures and extended ice cover can delay, and possible reduce the phytoplankton bloom at the base of the food web. Thus the NAO can directly affect primary productivity, which in turn may influence $\delta^{13}C$ levels (Rau *et al.* 1989; Fogel *et al.* 1992; Fogel and Cifuentes 1993; Goericke *et al.* 1994). Gomes *et al.* (1995) noted dramatic decreases in phytoplankton abundance in 1991, perhaps as a function of the NAO. Similar decreases in phytoplankton production in the Northeast Atlantic from 1970-80 were attributable to the NAO (Mann 1993). Depletions in ^{13}C would persist throughout the food chain. Thus for the limited data available, annual depletion of ^{13}C levels seen in this study could correspond to decreases in primary productivity, particularly as the major decrease in $\delta^{13}C$ occurs at the same time as documented decreases in phytoplankton biomass (Gomes *et al.* 1995; but see Mertz and Myers 1994).

Changes in phytoplankton distribution, abundance, and bloom timing can in turn affect the zooplankton, nekton and higher consumers that depend upon it. The 'match/mismatch' hypothesis proposed by Cushing (1975), in which successful cohort development of a species depends upon a specific timing of events, may be invoked by delays in the phytoplankton bloom. Furthermore, salinity and temperature effects may continue to affect the zooplankton bloom, thus effecting stratification of the water column and mixing of nutrients (Mann and Drinkwater 1994). Finally, changes in salinity and temperature might, at a physiological level, affect the successful spawning of certain species (Carscadden *et al.* 1989; Helbig *et al.* 1992; Shackell *et al.* 1994; Gomes *et al.* 1995; Narayanan *et al.* 1995). Not all data indicate that Northwest Atlantic fish spawning is tightly linked to the recently observed oceanographic variation (Myers *et al.* 1993; Mertz and Myers 1994). However, changes in the zooplankton and lower trophic level nektonic species populations may affect the prey availability to higher consumers such as whales, and thus potentially the average prey $\delta^{15}N$ levels.

Anthropogenic-driven annual variations in prey abundances and distributions may also have affected prey availability for humpback whales. It is well documented that many groundfish species stocks have recently collapsed because of excessive fisheries exploitation (Lear and Parson 1993; Hutchings and Myers 1994, 1995; Gomes et al. 1995; Myers et al. 1996). Most of these species (*Gadus morhua, Reinhardtius hippoglossoides*, etc.) are not prey for humpback whales, and thus their collapse does not directly affect humpback foraging. However, indirect multispecies effects such as those suggested in harp seal-fishery interactions have not been assessed (Anonymous 1997).

Thus, as a result of natural and anthrogenic influences, changes in abundance and distribution of other species may be affecting humpback isotopic composition by altering local availability of prey. Several potential prey species are known to have decreased in abundance or shifted in their distribution. In Newfoundland and Labrador waters, capelin are documented to have

moved southeast within this decade (Lilly 1994; Miller 1994a, 1994b). Data regarding capelin stock abundance are conflicting (for example, see Carscadden 1995). A collapse of offshore capelin stocks is thought to have induced an inshore movement of humpbacks in the early 1980s (Whitehead and Carscadden 1985; Whitehead and Glass 1985). Arctic cod stocks that were previously distributed on the Labrador shelf (Lear 1979) have also moved southeast (Lilly *et al.* 1994). Previously exploited herring stocks have failed to return to historical population sizes (Wheeler *et al.* 1994; Wheeler and Winters 1996). In other feeding substocks of various Northwest Atlantic balaenopterids, changes in spatial distribution are thought to be the results of prey distribution (Payne *et al.* 1990; Weinrich *et al.* 1997).

There seems little doubt that the availability of prey species to humpback whales has changed within the past decade, whether caused by natural events or by direct or indirect effects of stock exploitation (Gomes *et al.* 1995). Montevecchi and Myers (1996) have also noted changes in the fish diet of seabirds since 1977. Such changes in prey availability, and thus diet composition, can impact annual $\delta^{15}N$ values in humpbacks. Since 1990, annual $\delta^{15}N$ values have increased by ~1.5‰. Assuming f_N is 3‰, this suggests that humpbacks have moved up the trophic chain, taking prey items that are perhaps slightly larger, or perhaps different species. One obvious possibility with the changes in capelin stock distribution is the substitution of Arctic cod, which on average, ranks slightly higher on the trophic scale than capelin (Figure 3.4); alternatively, increased abundance in capelin might result in a higher intake of capelin in the humpback's diet. As a caution, however, explaining a change in $\delta^{15}N$ values as a function of differences in diet composition assumes $\delta^{15}N$ values of prey have remained constant. This assumption is addressed in Section 5.3.

In summary, annual changes in isotopic composition may be explained by examination of oceanographic and biological changes in the environment. Decreases in $\delta^{13}C$ could be the result of cold water influx into the Northwest Atlantic caused by the NAO, affecting primary productivity. Increases in $\delta^{15}N$ may be in response to changes in primary productivity, and/or

temporal, spatial or abundance shifts in prey species. Similar changes in isotopic composition were also seen for fish species in the Gulf of Maine by Wainwright *et al.* (1993).

5.3 Is the range of isotopic values in humpback whales supported by isotopic values in known prey?

If the isotopic fractionation between prey and consumer is known, then consumer CIGs should be predictable from a knowledge of prey isotopic composition. Examinations of Figures 3.4 and 4.15 demonstrate that a majority of humpbacks feed at TL4. Isotopic data from known prey of humpback whales suggests that capelin, herring and Arctic cod feed at ~TL3.5, with a number of plankton species feeding at TL3 (ctenophores, pterapods, and some species of euphausiids, mysids, copepods and amphipods). Thus, a mismatch is observed between the assumed prey of humpbacks, and the trophic level of humpbacks suggested by isotopic analyses. For example, the isotopic composition of humpbacks sampled in July was enriched relative to capelin by only ~0.5‰ ($\delta^{13}C$) and ~1.0‰ ($\delta^{15}N$).

The mismatch between prey and consumer in this study can be explained in several ways. First, the assumptions of f_N = 3‰ may be incorrect. This seems unlikely given that most studies of fractionation across trophic levels suggest otherwise (DeNiro and Epstein 1978, 1981; Minigawa and Wada 1984; Ostrom and Fry 1993), although it is acknowledged that these assessments are based on whole bodies or muscle tissue, not skin tissue. While it is accepted that f_N for whole animals is ~3‰ (DeNiro and Epstein 1981; Minigawa and Wada 1984), it is also known that differing fractionation and turnover rates between tissue types can alter this value (Tieszen *et al.* 1983; Hobson and Clark 1992b). Ames *et al.* (1996) noted unusually high $\delta^{13}C$ fractionation between sloughed skin of manatees and their diet. Abend and Smith (1995; 1997) suggest a $\delta^{15}N$ fractionation between skin and diet of 1.7‰. However, there are design flaws inherent in each of these studies. For example, one study uses captive animals, the other uses

data from net-entrapped animals. Ames *et al.* (1996) do not measure the behaviour of $\delta^{15}N$ values, and the somewhat high fractionation constant reported may be specific to sloughed (i.e. dead) tissue. Abend and Smith's (1995; 1997) diet $\delta^{15}N$ values are estimations of assumed prey.

The most applicable data to this study come from Hobson *et al.* (1996), who demonstrated a $\delta^{15}N$ fractionation between skin and diet of 2.3‰ for harp seals. Although it measures tissues taken from captive animals, Hobson *et al.* 's (1996) data represent the most comprehensive study to date of tissue fractionation in marine mammals. If one assumes an f_N of ~2.3‰ for humpback skin, the isotopic mismatch between prey and consumer is substantially reduced.

Second, previous assessments of humpbacks that used analysis of gut contents may be biased towards prey species whose hard parts are retained for identification. For example, when SIA was initially applied to foraging harp seals, $\delta^{15}N$ levels appeared too low to be caused by a predominance of fish in the stomach. Instead, smaller shrimp, previously underestimated by analysis of gut contents, may have been taken in greater proportions to account for the lower $\delta^{15}N$ levels (W. Montevecchi and K. Hobson unpub. data). Thus in this study, $\delta^{15}N$ levels suggest that humpbacks take higher proportions of low-trophic-level plankton that have previously been unquantified in analysis of gut contents (Nemoto 1970; Mitchell 1973, 1974; Kawamura 1980). An examination of Table 3.5 and Figure 3.4 suggests several other planktonic species at lower trophic levels (and therefore lower $\delta^{15}N$ and $\delta^{13}C$ values) that may be potential prey of humpbacks.

Third, if humpback isotopic composition has changed over the past 10 years (Section 5.2.4), it is possible that prey isotopic composition did also. To examine this hypothesis, annual data collected by standardized protocols are required; that is, within the same month and same geographic region, for prey of the same size and sex. Such data do not exist, although a nonparametric analysis of samples collected from 1984-1996 suggests that capelin $\delta^{15}N$ values may have increased over the same 10 year period that humpback $\delta^{15}N$ values increased (Figure 4.13). Extreme caution should be used in interpreting the longitudinal prey data; of the

two prey species measured (capelin and herring), and of the two isotopic variables measured for each, only $\delta^{15}N$ values for capelin increased significantly over the 10 year period. All four time series are incomplete, and none attempt to standardize data collection. For example, data from 1996 (J. Lawson and V. Lesage unpub. data) include animals ranging in length 105-155 mm, from two geographically distinct regions (Gulf of St. Lawrence and Labrador nearshore waters), collected in both January and August. Beyond problems of data standardization, Figure 4.13 suggests that $\delta^{15}N$ for at least one prey species of humpbacks has increased by <1‰ in the period 1988-1994. Concurrently, humpback $\delta^{15}N$ values have increased by almost 2‰. Thus increasing prey $\delta^{15}N$ values can only partly explain annual $\delta^{15}N$ increases in humpback whales.

Fourth, the isotopic composition of capelin reported here may not be an accurate assessment of populations in the wild. A capelin school will consist of different age classes that vary in strength annually. If capelin diet varies between age-classes, then the average isotopic signature of a capelin school will vary according to its age composition. Thus a population of capelin with predominantly 1-2 yr old capelin might have lower isotopic values than one containing more mature capelin. The current isotopic assessment of capelin does not address this concern, and more data is needed concerning the relationship between size of prey and its isotopic composition. In addition, since balaenopterid abundance is correlated with specific year classes of prey (Whitehead and Carscadden 1985), humpback whales may exhibit a year-class preference within capelin.

Finally, all prey specimens reported in this thesis were analyzed isotopically with their guts removed. In the wild, humpbacks will consume not only the prey item, but also the gut-contents of that prey item. This would effectively lower the consumer's isotopic signature, as it would be indirectly foraging upon lower trophic level species recently ingested by the prey.

This study emphasizes the need to collect concurrent prey data when conducting any SIA study of a consumer. In cases where this is done (e.g., J. Lawson unpub. data), excellent isotopic

separation can be achieved between prey species (Table 4.8c, Figure 4.14). For example, although capelin and Arctic cod are relatively inseparable with respect to $\delta^{15}N$ values, good separation can be seen with respect to $\delta^{13}C$ values. Squid and shrimp can be further delineated by examination of $\delta^{15}N$ levels (Figure 4.14).

5.4 Do differences exist between the Northwest Atlantic balaenopterids?

The two main balaenopterids sampled in this study—finback and humpback whales—could be separated on the basis of CIG (Figure 4.12). The only minke whale sampled in this study could also be isotopically separated from finback whales. Isotopic differences between balaenopterids are likely a function of diet composition. Data from this study and previous analyses of gut contents suggest that humpback whales are mainly piscivorous, although a proportion of their diet may include lower trophic level plankton species (Sergeant 1966; Nemoto 1970; Mitchell 1973, 1974; Kawamura 1980). Similarities between humpback and minke isotopic compositions suggest that minke whales have a similar diet to humpbacks. However, isotopic data suggests that the finbacks sampled in this study were more planktivorous in nature, feeding at $\leq$ TL3. This hypothesis is based on differences in $\delta^{15}N$ values between species. Although Figure 4.12 suggests significant differences between finbacks and humpbacks with respect to both isotopic variables, comparison of the finback data to humpbacks found in the same area and year (Figure 4.17) demonstrates that $\delta^{13}C$ ratios between the two species were not different.

Previously, assessments of finback diet were based mainly on analysis of gut contents sampled during whaling operations (Sergeant 1966; Mitchell 1974; Kawamura 1980). Such analyses indicated that Newfoundland finbacks were principally piscivorous, feeding on krill and copepods early in the season, but later capelin and herring. Possible krill and copepod species include *Thysanoessa inermis*, *Meganyctiphanes norvegica*, *Calanus finmarchicus*, and

Temora longicornis (Kawamura 1980). Finbacks caught off Nova Scotia appeared to be almost exclusively planktivorous, feeding on *M. norvegica* and *Thysanoessa* spp. (Mitchell 1974; Kawamura 1980). Thus Kawamura (1980) stressed the importance of euphausiids, specifically *M. norvegica*, in finback diet. Differences between Newfoundland and Nova Scotian finback diets were likely a function of prey availability (Mitchell 1974; Gambell 1985).

Anecdotal accounts of finbacks feeding on high density schools of herring, capelin and sand lance are available (Overholtz and Nicolas 1979; Watkins and Schevill 1979; Piatt and Methven 1992), and in Newfoundland, finback whales are often seen in association with capelin schools (Whitehead and Carlson 1988; Piatt *et al.* 1989), particularly 2-3 yr old immature capelin (Whitehead and Carscadden 1985). Such observations prompt the suggestion that humpback and finback whales might be competing for similar resources (Mitchell 1974; Meredith and Campbell 1988; Whitehead and Carlson 1988); as a result, it is also suggested that finbacks numbers may have declined (Lynch and Whitehead 1984; Whitehead and Carscadden 1985). However, finback whales may have a more offshore distribution in comparison to humpbacks, thus reducing competition (Lynch and Whitehead 1984; Piatt *et al.* 1989).

Evidence from this study further strengthens the argument of reduced competition between sympatric humpback and finback whales. In fact, for the small sample measured, data suggests that Newfoundland finbacks were entirely planktivorous. Isotopic data suggest that from the planktonic species listed by Kawamura (1980), only *M. norvegica* and *T. longicornis* would be likely dietary items (Table 2.5, Figure 2.4). These finbacks were sampled early in the season (DoY = 157–190), a time in which these animals are reported to be mainly planktivorous (Sergeant 1966). A switch to a piscivorous diet may have occurred later in the season. However, lack of temporal overlap between humpbacks and finbacks during the sampled period argues for further lack of competition between these two species early in the season.

These findings are in contrast to a previous SIA study comparing finback and humpback whales from the Gulf of St. Lawrence (Edds and Macfarlane 1987). Borobia *et al.* (1995) presented $\delta^{13}C$ values derived from the lipid quotient of blubber samples taken by non-lethal biopsy. They quoted a significant difference between the two species in $\delta^{13}C$, and thus suggested that humpbacks in the Gulf of St. Lawrence fed at a slightly lower level than finbacks. However, it should be noted that the difference found between the two species (0.3‰) and the standard deviations surrounding the means for each species (±0.4‰, ±0.5‰ respectively) closely approached the resolution of their measurement (±0.2‰).

Northwest Atlantic minke whales are cited as being mainly piscivorous (Stewart and Leatherwood 1985), feeding on similar prey to humpbacks, such as capelin and other schooling small fish, but perhaps at lower densities and lesser age classes (Mitchell 1974; Whitehead and Carscadden 1985; Piatt *et al.* 1989; Piatt and Methven 1992). Limited isotopic data presented in this study confirm that Newfoundland minke whales are feeding at similar trophic levels to humpback whales.

5.5 Use of SIA in assessing the role of balaenopterids within the Northwest Atlantic ecosystem

Balaenopterids and other marine mammals play a substantial role within ecosystems (Sergeant 1966; Mitchell 1974; Anonymous 1997; Sigurjónsson and Vikingsson in press; Stefánsson *et al.* in press). In other ecosystems such interactions have been partially modelled (Hain *et al.* 1992; Markussen *et al.* 1992a; Sigurjónsson and Vikingsson in press; Stefánsson *et al.* in press), but Northwest Atlantic species remain unquantified. For example, analysis of interactions between fisheries and perhaps the best studied marine mammal in the Northwest Atlantic—the harp seal—indicates that further data are still required for even extensively studied species (Anonymous 1997). Such models depend upon accurate assessments of diet. This study has used

SIA to assess trophic position for several balaenopterids, and has demonstrated that considerable variability occurs within and between species. It has highlighted the need to account for seasonal and annual changes in diet.

Many authors have documented the recent biotic and abiotic changes in the Northwest Atlantic ecosystem. Data from this study suggest that humpback whales have also been affected by these changes, responding with possible switches in diet. This study demonstrates the inexorable link between balaenopterids and the remaining ecosystem, and thus indicates areas that should be further studied in modelling species interactions within the Northwest Atlantic ecosystem.

6. Conclusions and Recommendations

Use of biopsy samples as a tissue source for stable-isotopic analysis provides a non-lethal alternative to analysis of gut contents in studies of diet. This study first demonstrated that skin samples showed similar isotopic composition to muscle tissue. However, the integration (turnover) time of skin tissue was considerably shorter than muscle; later data from this study suggested that isotopic ratios in skin tissue reflected diet taken within the previous 7–14 days.

This study measured the isotopic range of $\delta^{13}C$ ($\bar{x} \pm SD$; -18.5 ± 0.28‰, n = 98) and $\delta^{15}N$ (14.6 ± 0.94‰, n = 106) for humpback whales on their summer feeding grounds. Large variability existed in both variables, likely a function of measurement precision (±0.2‰), unquantifiable within-individual sampling error, individual differences in physiological state, differences in diet, and differences in the isotopic composition of diet.

Little variability was seen between sexes, although anecdotal evidence suggested that lactating females would be isotopically distinguishable because of their unique biochemistry. Small isotopic variation between geographic areas were also found. These could be due to two factors: a) differences in oceanography between regions may alter fundamental isotopic ratios at the base of the food chain (i.e., phytoplankton); and b) humpbacks in different regions might have slightly different diets based on local prey availability. Evidence was found to support both of these hypotheses.

Differences in isotopic composition within a feeding season was thought to reflect prey availability. For example, peaks in isotopic variables in July were likely a function of the seasonal influx of capelin to the inshore. Substantial interannual isotopic variation was seen, suggesting an increase of 0.5TL over the past decade. Correspondingly, $\delta^{13}C$ ratios dropped dramatically over the same period. Large scale oceanographic and biological causes for these trends were considered. Isotopic evidence suggested that primary productivity may have decreased in the early 1990s as a result of a positive NAO (North Atlantic Oscillation) index.

The persistence of cold, less saline waters with greater periods of extensive ice cover may have also shifted prey distributions, thus altering prey compositions available to foraging humpbacks.

A slight mismatch between humpbacks and their prey species prompted an examination of the assumptions of the SIA model. It was concluded that humpbacks may be taking more planktonic prey than was previously thought, although it was also noted that the assumed value of f_N, the fractionation constant for $\delta^{15}N$ per trophic level, might be inappropriate for use with cetacean skin. Several other possible causes of the mismatch were also noted, including the varying isotopic composition of prey, and the indirect assimilation of lower-trophic level items retained within the guts of prey.

Isotopic comparison of humpbacks with two other balaenopterids suggested that species could be separated on the basis of $\delta^{15}N$ levels. Isotopic data formed a continuum between plankton-specialists and fish-specialists. Data for all balaenopterids measured in this study are plotted with other SIA data for balaenopterids of the Northwest Atlantic in Figure 6.1. Using SIA data, finback and blue whales are classified as planktivorous, and humpback and minke whales are classified as being more piscivorous.

Recommendations for future studies can be made. First, further data are needed on turnover rate, homogeneity, and fractionation aspects for cetacean tissues. Possible studies that may address some of these concerns would include:

- Multiple samples of different tissues taken from same individuals at different locations on the body.
- Isotopic monitoring of tissues in captive marine mammals (e.g., sloughed skin, blood) during manipulated changes in isotopically separable diets.
- Simultaneous application of gut-content and stable-isotopic analyses to hunted animals, sampling a variety of tissues and prey.

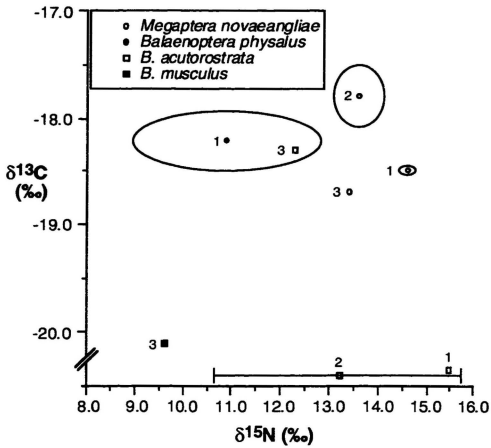


Figure 6.1. A review of isotopic values for northwestern Atlantic balaenopterids measured to date. Species CIGs are plotted with 95% confidence ellipses. Data come from (1) this study, (2) P. Ostrom and J. Lien unpub. data, (3) Ostrom *et al.* 1992. Note that isotopic values from study (3) are for single specimens. Minke whale data from studies (1) and (2) have no equivalent $\delta^{13}C$ values, and thus are represented at the base of the graph. Data from study (1) were obtained non-lethally from skin biopsies. All other data were obtained *post mortem* from muscle tissue. Planktivorous species (feeders at low trophic levels) are represented to the left of the graph; Piscivorous species (feeders at high trophic levels) occupy the right of the graph. Finback and blue whales are thus classified as planktivorous. Humpback and minke whales are classified as piscivorous.

- Stable-isotopic analyses of wintering humpbacks, both stranded and free-ranging.
- Concurrent measurement of sloughed and biopsied skin from free-ranging individuals.

Second, it was noted that this study only measured humpbacks on their summer grounds. Thus to complete the isotopic classification of this species, humpbacks should be sampled in other parts of their annual cycle. Potential studies would include:

- Application of SIA to wintering humpbacks, with special attention to pregnant and lactating individuals. This analysis could be performed in conjunction with fatty acid analysis and monitoring of hormone levels in skin biopsy samples (after techniques developed by Yoshioka *et al.* 1994).
- Longitudinal resampling of the same individuals throughout an annual cycle to determine variation in isotopic composition during feeding and fasting periods. This may also be achieved through the analysis of metabolically inert tissues, such as baleen, which are deposited on a yearly basis (Schell *et al.* 1989a).

Third, several limitations arose from the current study owing to the lack of substantial prey data. In future SIA studies of marine mammals, it is imperative that concurrent SIA studies of potential prey be also included on similar temporal and spatial scales (including assessment of particulate organic matter). In addition, an assessment of prey availability can be useful in determining diet selection.

Fourth, the long-term trends in $\delta^{13}C$ and $\delta^{15}N$ in humpbacks and other marine species in the Northwest Atlantic should continue to be monitored. To an extent, these isotopic ratios can be used to monitor ecosystem status and can be useful indicators to ecosystem managers.

Fifth, isotopic knowledge of other balaenopterids and large cetaceans should be extended. This thesis proposes a non-invasive technique that can be applied to all large cetaceans; with

some modifications, it may also be used on smaller cetaceans also (for example, using a less powerful delivery of the biopsy dart).

Finally, data from such studies should be distributed to marine managers as they construct models that assess marine mammal interactions within the ecosystem. To date, the role of cetaceans in the ecosystem has not been extensively examined. As ocean resources are further exploited these data will become increasingly important.

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Appendix A: Previous estimates of stable isotopic values for various marine mammals

Table A.1. Values of $\delta^{13}C$ and $\delta^{15}N$ (‰) for marine mammals (generic and species names are standardized from Jefferson *et al.* 1993). Values are given as $\bar{X} \pm SD$. In cases where these statistics are not available, ranges are given. An asterisk (*) is used for captive studies; in such cases isotopic values may not be representative of wild populations. Sources not using lipid-extracted (or lipid-corrected) values are indicated by a (†). In such instances, ranges and means quoted may have lower $\delta^{13}C$ values, and show greater variance, than in the case of lipid-extracted values.

Common name	Scientific name	*n*	Tissue	$\delta^{13}C$	$\delta^{15}N$	Source
Phocidae						
Harp seal	*Phoca groenlandica*	13	muscle	-	16.1 ± 1.2	Montevecchi *et al.* 1992
		1	muscle[a]	-19.0	15.5	Hobson *et al.* 1996[1]
		13	liver	-	16.5 ± 1.2	Montevecchi *et al.* 1992
		1	liver*	-19.7	16.1	Hobson *et al.* 1996
		5	skin*	-17.5 ± 0.5	15.3 ± 0.9	" "
		14	blood[2]*	-18.6 ± 0.4	14.7 ± 0.6	" "
		1	kidney*	-19.0	15.7	" "
		1	bone	-15.2	15.6	Schoeninger and DeNiro 1984
Bearded seal	*Erignathus barbatus*	4	muscle	-16.6 ± 0.5	16.8 ± 0.2	Hobson and Welch 1992
Ringed seal	*P. hispida*	9	muscle	-17.3 ± 0.7	17.3 ± 1.1	" "
		27	muscle	-18.1 ± 0.2	-	" "
		27	fat	-26.1 ± 0.2	-	" "
		27	bone	-16.2 ± 0.3	-	" "
Harbour seal[3]	*P. vitulina*	9	muscle	- 17.6 ± 0.2	18.6 ± 0.3	Hobson *et al.* 1997
		5	muscle	-17.1 ± 0.3	16.4 ± 0.2	" "

(Table 1, continued)

Common name	Scientific name	*n*	Tissue	$\delta^{13}C$	$\delta^{15}N$	Source
Phocidae (continued)						
Harbour seal	*P. vitulina*	3	bone	-12.3 ± 1.44	17.6 ± 1.65	Schoeninger and DeNiro 1984
Northern Fur seal	*Callorhinus ursinus*	7	muscle	-18.9 ± 0.4	16.6 ± 0.5	Hobson *et al.* 1997
Antarctic fur seal[4]	*Arctocephalus gazella*	7	muscle	-25 – -24.5	7.2 – 8.5	Rau *et al.* 1992
Ross seal[4]	*Ommatophoca rossii*	2	muscle	-22.9	9.1 – 9.3	" "
Crabeater seal[4]	*Lobodon carcinophagus*	15	muscle	-26 – -23	5.2 – 6.5	" "
Leopard seal[4]	*Hydrurga leptonyx*	5	muscle	-24.5 – -24	7.1 – 8.6	" "
Otariidae						
Californian sea lion	*Zalophus californianus*	4	bone	-12.6 ± 1.10	19.3 ± 2.65	Schoeninger and DeNiro 1984
Steller sea lion	*Eumetopias jubatus*	13	muscle	17.5 ± 0.2	-18.2 ± 0.2	Hobson *et al.* 1997
Odobenidae						
Walrus	*Odobenus rosmarus*	3	bone	-13.9 ± 0.25	12.3 ± 0.21	Schoeninger and DeNiro 1984
		6	muscle	-17.8 ± 0.3	12.5 ± 0.6	Hobson and Welch 1992

(Table 1, continued)

Common name	Scientific name	*n*	Tissue	$\delta^{13}C$	$\delta^{15}N$	Source
Odontoceti						
Short-finned pilot whale	*Globiocephala macrorhynchus*	3	bone	-12.1 ± 0.21	16.3 ± 0.55	Schoeninger and DeNiro 1984
Long-finned pilot whale	*G. melas*	9	skin	-18.8 ± 0.1[5]	13.2 ± 1.1	Abend and Smith 1995a†
		9	muscle	-18.0 ± 0.1[5]	12.7 ± 0.9	" "
		9	blubber	-	15.2 ± 0.8	" "
		9	teeth	-	14.0 ± 0.8	" "
Narwhal	*Monodon monoceros*	4	muscle	-18.0 ± 0.4	15.8 ± 0.7	Hobson and Welch 1992
Common dolphin	*Delphinus delphis*	1	muscle	-17.8	14.8	Ostrom *et al.* 1992†
		3	bone	-13.9 ± 0.90	15.6 ± 0.90	Schoeninger and DeNiro 1984
Pacific white-sided dolphin	*Lagenoryhnchus obliquidens*	3	bone	-12.2 ± 0.42	15.1 ± 0.85	" "
Bottlenose dolphin	*Tursiops truncatus*	2	bone	-12.5 ± 1.20	16.2 ± 1.41	" "
Dall's porpoise	*Phocoenoides dalli*	3	bone	-12.9 ± 0.52	15.6 ± 0.85	" "
Harbour porpoise	*Phocoena phocoena*	1	bone	-11.6	16.6	" "

(Table 1, continued)

Common name	Scientific name	*n*	Tissue	$\delta^{13}C$	$\delta^{15}N$	Source
Odontoceti (continued)						
White-beaked dolphin	*L. albirostris*	1	muscle	-18.1	16.2	Ostrom *et al.* 1992†
Sperm whale	*Physeter macrocephalus*	1	muscle	-22.8	11.1	" "
Beluga whale	*Delphinapterus leucas*	1	muscle	-17.6	13.6	Ostrom *et al.* 1992†
		6	muscle	-18.1 ± 0.5	16.6 ± 0.6	Hobson and Welch 1992
		2	bone	-14.1 ± 0.21	16.9 ± 1.34	Schoeninger and DeNiro 1984
Pygmy sperm whale	*Kogia breviceps*	1	muscle	-17.2	11.9	Ostrom *et al.* 1992†
Sowerby's Beaked whale	*Mesoplodon bidens*	4	muscle	-18.5 ± 0.5	11.7 ± 0.6	" "
Mysticeti						
Bowhead whale	*Balaena mysticetus*	3	bone	-15.9 ± 0.57	14.5 ± 0.81	Schoeninger and DeNiro 1984
		12	muscle[4]	-19.2 – -21.5	-	Schell *et al.* 1989†
		10	visceral fat[4]	-24.4 – -27.7	-	" "
		16	baleen[4]	-15.0 – -23.0	-	" "

(Table 1, continued)

Common name	Scientific name	*n*	Tissue	$\delta^{13}C$	$\delta^{15}N$	Source
Mysticeti (continued)						
Blue whale	*Balaenoptera musculus*	1	muscle	-20.1	9.6	Ostrom *et al.* 1992†
		1	unknown[6]	-17.6	-	Rau *et al.* 1983†
		2	bone	-13.6 ± 1.34	13.8 ± 2.90	Schoeninger and DeNiro 1984
Humpback whale	*Megaptera novaeangliae*	1	muscle	-18.7	13.4	Ostrom *et al.* 1992†
		10	blubber	-25.8 ±0.5	-	Borobia *et al.* 1995†
Finback whale	*Balaenoptera physalus*	19	blubber	-25.5 ± 0.4	-	" "
		1	bone	-15.4	12.7	Schoeninger and DeNiro 1984
Minke whale	*B. acutorostrata*	1	muscle	-18.3	12.3	Ostrom *et al.* 1992†
		2	bone	-14.0 ± 1.98	14.4 ± 0.78	Schoeninger and DeNiro 1984
Gray whale	*Eschrichtius robustus*	2	bone	-13.3 ± 0.42	13.0 ± 1.41	" "
Sirenia						
West Indian manatee	*Trichechus manatus*	20	skin	-16.5 ± 5.9	-	Ames *et al.* 1996†
		20	blubber	-18.3 ± 3.2	-	" "
		20	liver	-16.3 ± 4.0	-	" "

(Table 1, continued)

Common name	Scientific name	*n*	Tissue	$\delta^{13}C$	$\delta^{15}N$	Source
Sirenia (continued)						
West Indian manatee	*T. manatus*	20	kidney	-16.8 ± 3.3	-	Ames *et al.* 1996†
		3	skin*	-25.0 – -32.0	-	" "
Ursidae						
Polar bear	*Ursus maritimus*	12	muscle	-17.7 ± 0.2	-	Ramsay and Hobson 1991
		3	muscle	-18.0 ± 0.6	21.1 ± 0.6	Hobson and Welch 1992
		12	fat	-24.7 ± 0.1	-	Ramsay and Hobson 1991
		12	bone	-15.7 ± 0.1	-	" "
		56	plasma	-19.6 – -19.9	-	Hobson and Stirling 1997
		31	cellular blood	-18.1 – -18.2	-	" "
Mustelidae						
Sea otter	*Enhydra lutris*	3	bone	-9.8 ± 0.21	14.2 ± 1.08	Schoeninger and DeNiro 1984

Notes for Table 1.1

[1]Hobson *et al.* (1996) present data for 11 tissue types; for conciseness, only six are presented here.

[2]Sample incorporates whole blood and did not distinguish between plasma and cellular fractions

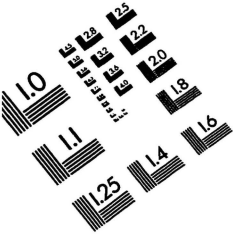
[3]The two sets of values presented by Hobson *et al.* (1997) represent samples taken from Alaska and Washington, respectively.

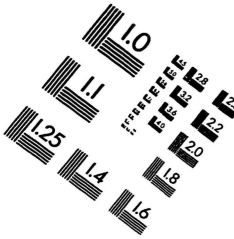
[4]Delta values extrapolated from graphs, and are approximate.

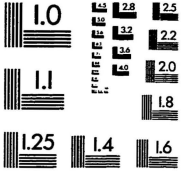
[5]Values of $\delta^{13}C$ were obtained from only 3 individuals.

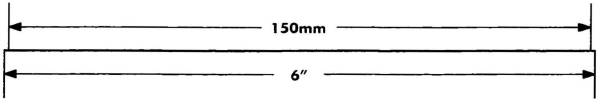
[6]This sample is probably derived from skin tissue, given the nature of the encounter.

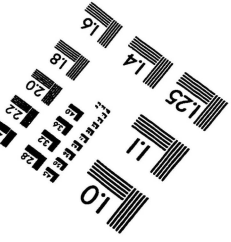
IMAGE EVALUATION
TEST TARGET (QA-3)











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