

Diet of northern bottlenose whales inferred from fatty-acid and stable-isotope analyses of biopsy samples

Sascha K. Hooker, Sara J. Iverson, Peggy Ostrom, and Sean C. Smith

Abstract: The Gully submarine canyon off eastern Canada has been designated a pilot marine protected area largely because of the northern bottlenose whales (*Hyperoodon ampullatus*) found there. Studies of this species' diet elsewhere in the North Atlantic Ocean have suggested specialization on the deep-sea squid *Gonatus fabricii*. We found a high proportion of the congener *Gonatus steenstrupi* in the stomachs of two bottlenose whales stranded in eastern Canada. In 1997, we collected remote biopsy samples from free-ranging bottlenose whales off Nova Scotia; fatty acids were determined from blubber samples and stable isotopes (carbon and nitrogen) from skin samples. Although fatty-acid stratification throughout the depth of the blubber layer was present (determined from blubber samples of stranded animals), the magnitude of stratification was less pronounced than in many other cetaceans, allowing some qualitative inferences to be made from shallow biopsy samples. Fatty-acid patterns and stable-isotope values from whales were compared with those in samples of *G. fabricii* from the Norwegian Sea. Blubber fatty acid composition was similar in characteristics to that of adult *G. fabricii* but was markedly distinct from that of juvenile *G. fabricii* and other recorded prey species. Nitrogen-isotope values implied that bottlenose whales (mean 15.3‰) and adult *G. fabricii* (mean 13.7‰) occupy high trophic levels. Overall, the results of these techniques concurred in suggesting that squid of the genus *Gonatus* may form a major part of the diet of bottlenose whales in the Gully.

Résumé : Le canyon sousmarin Gully, au large de la côte est du Canada, a été désigné zone protégée en grande partie à cause de la présence des Baleines-à-bec communes (*Hyperoodon ampullatus*). L'étude du régime alimentaire de cette espèce ailleurs dans le nord de l'Atlantique indique une préférence pour le calmar d'eau profonde *Gonatus fabricii*. Nous avons constaté la présence d'une forte proportion de *Gonatus steenstrupi*, une espèce congénère, dans les estomacs de deux baleines échouées dans l'est du Canada. En 1997, nous avons recueilli à distance des biopsies de baleines en nature au large de la Nouvelle-Écosse; les acides gras ont été déterminés dans les échantillons de lard et les isotopes stables (carbone et azote) dans les échantillons de peau. Bien qu'il y ait stratification des acides gras dans toute la couche de lard (déterminée dans des échantillons de lard prélevés sur des baleines échouées), l'importance de la stratification est moins prononcée que chez plusieurs autres cétacés, ce qui permet de faire des suppositions qualitatives à partir des biopsies faites en eau peu profonde. La distribution des acides gras et la valeur des isotopes stables des baleines ont été comparées à celles de *G. fabricii* prélevés dans la mer de Norvège. La composition en acides gras du lard est semblable par certaines caractéristiques à celle des adultes de *G. fabricii*, mais est nettement distincte de celle des *G. fabricii* juvéniles et à celle d'autres espèces de proies. La valeur des isotopes d'azote indique que les Baleines-à-bec communes (moyenne 15,3 ‰) et les adultes de *G. fabricii* (13,7 ‰) occupent des niveaux trophiques élevés. Dans l'ensemble, ces techniques s'accordent pour indiquer que les calmars du genre *Gonatus* constituent probablement une partie importante du régime alimentaire des Baleines-à-bec communes dans le Gully.

[Traduit par la Rédaction]

Introduction

Knowledge of the diet of any species is fundamental to understanding its ecology. The northern bottlenose whale (*Hyperoodon ampullatus*) is a member of the beaked whale family (Ziphiidae), probably the least known family of large

mammals. Although bottlenose whales have received more attention than other beaked whale species because they were heavily hunted, knowledge of their ecology remains poor (Reeves et al. 1993). The species is found only in the North Atlantic Ocean, and their preference for a deep-water habitat, thus often far from shore, makes them difficult to study. These whales are consistently found above a large submarine canyon known as the Gully, 200 km off the east coast of Canada (44°N, 59°W). In December 1998, the Gully was designated a pilot marine protected area largely on account of this population of bottlenose whales. The high use of the region by these whales and their apparent reliance on it (Whitehead et al. 1997) are assumed to be a result of the particular food supply found there. However, the prey composition of the diet of bottlenose whales in the Gully is essentially unknown, as they feed at depths of over 800 m

Received September 11, 2000. Accepted June 13, 2001.
Published on the NRC Research Press Web site at
<http://cjz.nrc.ca> on August 10, 2001.

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(Hooker and Baird 1999) and sampling potential prey species at such great depths in this topographically diverse region presents extreme methodological difficulties.

Previously published data on the diet of northern bottlenose whales have come primarily from the northern and eastern North Atlantic Ocean and from either hunted or beach-cast animals. Ohlin (1893) reported that bottlenose whales are teuthophagous, feeding primarily on "cuttle-fishes," but that the stomachs of some specimens contained an abundance of herring (*Clupea harengus*). There is wide agreement throughout the literature that the primary squid species found in the diet is *Gonatus fabricii* (Benjaminsen and Christensen 1979; Clarke and Kristensen 1980; Lick and Piatkowski 1998). However, there is some evidence of dietary differences between populations of bottlenose whales. Benjaminsen and Christensen (1979) examined the stomach contents of bottlenose whales caught off Iceland ($n = 46$) and Labrador ($n = 108$). *Gonatus fabricii* was the major dietary component in both locations, but 50% of whales off Labrador also contained fish remains, while only 10% of those off Iceland had eaten any fish. Three stranded bottlenose whales in the North Atlantic (one in the Faeroe Islands and one in Jutland, Denmark (Clarke and Kristensen 1980) and one in Hiddensee Island in the Baltic Sea (Lick and Piatkowski 1998)) contained predominantly (or only) *G. fabricii* remains in their stomachs. No stomach contents from bottlenose whales stranded or taken by whalers in eastern Canada have previously been documented.

A problem with dietary studies based on stomach contents of stranded animals is the inherent delay between feeding and the stranding event. Although collection of fecal samples is another means of investigating diet (cf. sperm whales, *Physeter macrocephalus*; Smith and Whitehead 2000), we have been unable to collect fecal samples from bottlenose whales in the Gully, despite considerable efforts. Furthermore, both stomach-content and fecal analyses provide detail on only the most recent few meals and may be biased because of the increased retention of certain less digestible dietary items (such as fish otoliths and squid beaks) (Bigg and Fawcett 1985; Smith and Whitehead 2000). Recently, two new techniques (fatty acid signature analysis and stable-isotope analysis) have been used to assess aspects of diet using blubber and skin samples, respectively (Iverson 1993; Iverson et al. 1997; Todd et al. 1997). These methods are based on the principle that the patterns of fatty acids and the composition of stable isotopes in an animal's diet will be reflected in its tissues (Klem 1935; Ackman and Eaton 1966; Rouvinen and Kiiskinen 1989; Hobson 1990; Wada et al. 1991; Iverson et al. 1995; Kirsch et al. 1998, 2000). Thus, compared with the immediacy of the record provided by stomach contents, interpretation of diet using these analyses is based on tissue samples that reflect the average composition of food resources that have been assimilated over periods of days to months (Tiezen 1978; Kirsch et al. 1998, 2000).

In marine mammals the blubber layer is the most important site of fat storage and can be used to assess differences in diets of individuals (Iverson et al. 1995, 1997). While biopsy sampling of live cetaceans is widely used to collect skin and blubber samples (International Whaling Commission 1989), such biopsies usually sample only the outer 1.5–2.5 cm

of the blubber layer (often less than one-quarter of the blubber thickness). In many cetaceans the most metabolically active part of the blubber, where most active deposition and withdrawal of lipids likely take place, appears to be the middle or inner layers adjacent to the muscle (Lockyer et al. 1984; Koopman et al. 1996; Koopman 2001). Thus, the high degree of stratification in the blubber layer often precludes the use of outer blubber samples for diet evaluation (S.J. Iverson, personal observation, and H.N. Koopman, personal communication). Therefore, in order to use fatty-acid signatures observed in the outer blubber of bottlenose whales to make inferences about diet it is first necessary to evaluate the degree of stratification of fatty-acid and lipid-class composition across the blubber layer.

Finally, both fatty acid signature analysis and stable-isotope analysis provide information about differences between individuals and populations, but can only allow diet to be interpreted through a comparison with patterns in their prey. Unfortunately, deep-water sampling for prey in the Gully canyon (where the whales appear to feed; Hooker and Baird 1999) is extremely problematic. In addition to the canyon's depth (1500–2000 m in the centre), there is a high likelihood of snaring trawl nets on the steep walls. Our strategy, therefore, was to compare the stable-isotope and fatty-acid values from bottlenose whales, nine samples of *G. fabricii* obtained from the Norwegian Sea, and other potential prey species (herring and redfish, *Sebastes* sp.) collected directly from the Gully region (S.J. Iverson, W.D. Bowen, and R.G. Ackman, unpublished data). We were fortunate to obtain the *G. fabricii* samples because, like those of the Gully, the depths (1100 m) and topographic characteristics of the Norwegian Sea offer an extreme challenge for sampling. Given the remoteness of these whales and the problems involved in sampling in the Gully and other deep ocean sites, our results, despite the small sample size, provide insight that would otherwise be extremely difficult to achieve.

The aims of our study were (i) to document the stomach contents of two animals stranded in eastern Canada and potentially from the Gully population, (ii) to investigate whether fatty-acid data obtained from blubber biopsies of bottlenose whales might be used to infer aspects of diet, and (iii) to assess, using fatty acid signature analysis and stable-isotope analysis, aspects of the diet of bottlenose whales in the Gully, particularly in relation to their suspected primary prey, squid of the genus *Gonatus*.

Methods

Sample collection

Samples were collected from three whales that stranded in eastern Canada (Table 1). Stomach contents of two of these whales were collected, frozen, and later transferred to ethanol for storage and identification. No fish remains were found. The squid beaks contained in these stomachs were sorted into upper and lower beaks, and lower beaks were further sorted into genus and species (cf. Clarke 1986). Full blubber cores, which were necessary to compare differences in lipid classes and fatty acids across the blubber layer, were taken from all three stranded animals and frozen in aluminum foil. A small (approximately 0.5 cm in diameter \times 5 cm long) full-depth blubber core was then taken from each of these and was divided into three equal pieces: inner (next to the muscle layer), middle, and outer (next to the skin).

Table 1. Stranded northern bottlenose whales (*Hyperoodon ampullatus*) from which full-blubber samples were used for analysis of fatty-acid stratification.

Bottlenose whale code	Date	Location	Age and sex	Note
Ha-01-92 ^a	8 Oct. 1992	Sydney, N.S.	Subadult male	Observed for 11 days prior to stranding
Ha-02-94 ^b	6 Nov. 1994	Tadoussac, Que.	Infant male (suckling)	
Ha-97-01 ^c	8 Sept. 1997	Sept-Îles, Que.	Subadult male	

^aSample collected by Nova Scotia Stranding Network, Halifax.

^bSample collected by Canadian Cooperative Wildlife Health Centre, College of Veterinary Medicine, University of Montreal, Que.

^cSample collected by Department of Fisheries and Oceans, Mont-Joli, Que.

Biopsy samples ($n = 18$) were collected from free-ranging northern bottlenose whales in the Gully (44°N, 59°W) on 16 July, 12–14 August, and 16 August 1997. The biopsy dart was made up of a crossbow bolt to which was attached a solvent-cleaned hollow stainless-steel biopsy tip, 2.5 cm long and 0.6 cm in diameter (cf. Barrett-Lennard et al. 1996). A 12 m long auxiliary sailing vessel was maneuvered alongside a whale at distances ranging from 5 to 15 m, and the dart was fired from a 67 kg draw crossbow (Barnett Wildcat XL) at the midlateral region near the dorsal fin of the whale. A stop collar attached to the tip of the bolt prevented penetration deeper than the biopsy tip and caused the bolt to rebound upon impact with the whale. The darts were designed to float and were collected using a dip net. The blubber and skin were then separated. Blubber samples (for fatty-acid analysis) approximately 1.5 cm long \times 0.2 cm in diameter were immediately placed in solvent-rinsed glass vials containing several millilitres of 2:1 chloroform:methanol with 0.01% butylated hydroxytoluene (BHT). Skin samples (for stable-isotope analysis) approximately 0.2 cm long \times 0.4 cm in diameter were wrapped in aluminum foil and placed in a dry shipper filled with liquid nitrogen.

In the field we attempted to avoid sampling the same animal twice (by keeping track of the scar characteristics of biopsied whales), and whenever possible a photographic record was kept of a biopsied whale. Ten of the 17 animals sampled for fatty acids and stable isotopes were identified photographically, and the sex of all samples was determined genetically (Gowans et al. 2000). We are confident that no animal was sampled twice within a period of a few days, but there is a small chance that an animal sampled in July may have been sampled again in August (representing, at most, 2 duplicate samples). Bottlenose whales are sexually dimorphic, so adult males can be identified in the field (or from photographs) on the basis of their size and lighter coloured, flattened forehead (Gray 1882). This provides a means to establish the maturity of males.

No samples of the primary prey species expected from the Gully (*Gonatus steenstrupi*; see Results) were available. However, we obtained samples of *G. fabricii* (the suspected primary prey of bottlenose whales elsewhere in the North Atlantic) for comparative purposes. Nine samples of *G. fabricii* were available from a collection made farther north in the Atlantic Ocean (the Norwegian Sea) sampled at depths of approximately 1100 m in July 1996 (see Bjørke et al. 1997). Although differences in fatty-acid composition in the genus *Gonatus* may occur across regions, these samples should still provide an index of their general fatty acid patterns (e.g., see Iverson et al. 1997). These samples were stored frozen until analysis. Three adult and six juvenile specimens of *G. fabricii* were individually homogenized and weighed and subsamples were placed in solvent (for fatty-acid analysis) or frozen (for stable-isotope analysis).

Lipid-class and fatty-acid analysis

Fatty-acid analysis of all samples was carried out following

methods described in Iverson et al. (1997). In brief, lipids were extracted using a modified Folch extraction (Folch et al. 1957). Lipid-class composition of inner, middle, and outer blubber from the stranded animals was analysed by H.N. Koopman (Duke University, Durham, N.C., U.S.A.) using Iatroscan (quantitative thin-layer chromatography with flame-ionization detection (TLC-FID); for details of the technique see Ackman and Heras 1997). Total extracted lipids were suspended in hexane at 15 mg/mL. One microlitre of sample was spotted onto each of three rods (Chromarod III – silica gel) and developed in a solvent system of 96:4:1 hexane : ethyl acetate : formic acid for 48 min. Following incubation, rods were dried and then analysed by TLC-FID Iatroscan. Lipid-class concentrations were calculated using standard curves and converted into percentages of total lipid.

Fatty acid methyl esters were prepared directly from the pure extracted lipid and then extracted into hexane. Analysis of fatty acid methyl esters was carried out using temperature-programmed gas-liquid chromatography. Fatty acids and isomers were identified from various sources, including quantitative standards (Nu Check Prep., Elysian, Minn.) and gas chromatography – mass spectrometry (Iverson et al. 1997). Individual fatty acids are expressed as mass percent of total fatty acids and are designated by shorthand IUPAC nomenclature of carbon chain length : number of double bonds and location ($n - x$) of the double bond nearest the terminal methyl group. Lipids from two blubber samples were also converted to fatty acid butyl esters to determine whether isovaleric acid (5:0) is present in this species (for exact methods see Koopman et al. 1996), as this component can represent up to 27% of total fatty acids in the outer blubber of some cetacean species and is lost with preparation of methyl esters (Koopman et al. 1996).

Since our primary interest was to interpret the results with respect to potential diet, the fatty acids selected for statistical comparison were those either known to be of dietary origin (Iverson 1993) and (or) present in fairly large quantities (>1%) in either bottlenose whale or *G. fabricii* samples (16:0, 18:0, 18:1 $n-9$, 20:1 $n-11$, 20:1 $n-9$, 20:5 $n-3$, 22:1 $n-11$, 22:1 $n-9$, and 22:6 $n-3$, hereafter referred to as major fatty acids). Fatty-acid compositions of two other potential prey species, herring ($n = 53$) and redfish ($n = 19$) from the Scotian Shelf (S.J. Iverson, W.D. Bowen, and R.G. Ackman, unpublished data), were included for reference in these comparisons.

Stable-isotope analysis

Skin samples were freeze-dried, ground, lipid-extracted, and ground again into a homogeneous fine powder. Lipids were removed by Soxhlet extraction using an azeotropic mixture of chloroform and methanol for 7 h. Lipid-extracted samples were analysed using a Carlo Erba elemental analyser interfaced to a Prism (Micromass) mass spectrometer (following methods described in Todd et al. 1997). Homogenized samples of three adult and two juvenile *G. fabricii* were also analysed using the same procedure.

Stable carbon- and nitrogen-isotope ratios are expressed as

$$\delta^{13}\text{C} \text{ or } \delta^{15}\text{N} (\text{‰}) = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 10^3$$

where R is $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$ for $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$, respectively. The standard for carbon was the Vienna-Peedee belemnite (V-PDB) and that for nitrogen was atmospheric nitrogen. Reproducibility associated with our isotope analysis was 0.2‰.

Statistical analysis

Since fatty-acid compositions are presented as percentages, all data were arcsine square root transformed and analysed using MANOVA tests. To explore the similarities and differences between samples, each sample was plotted for the first two principal components of the transformed major fatty acids listed above; t tests were used to compare differences in stable-isotope ratios and lipid contents of samples. All data are presented as means \pm 1 SEM unless otherwise indicated.

Results

Stomach contents and sample collection

All three whales sampled for stomach contents and full-blubber cores had live-stranded prior to death and were assessed as being in reasonably good body condition at the time of examination. The cause of death was not established for either of the subadult males; the infant was thought to have died following the loss of its mother (Table 1). Stomach contents were collected from both subadults. Both of these contained predominantly beaks of *G. steenstrupi* (identified according to Clarke 1986; Table 2). Of the squid species identified from all beaks, *Gonatus steenstrupi* were also the largest in mass (cf. Clarke and Kristensen 1980). Other common squid species found in these stomachs included *Taonius pavo*, *Teuthowenia* sp., and *Histioteuthis reversa*.

Eighteen biopsy samples were collected from free-ranging bottlenose whales during June–August 1997. Of these, blubber from 16 samples was retained for fatty-acid analysis and skin from 17 samples was retained for stable-isotope analysis (Table 3). Nine samples of *G. fabricii*, collected from the Norwegian Sea, were analysed. Three of these were adult squid: two females (mantle lengths 26.0 and 30.2 cm) and one male (mantle length 21.5 cm). These were processed for both fatty acids and stable isotopes. The small size of the six juvenile squid prevented subsampling, so four were processed for fatty acids and two for stable isotopes (Table 4).

Lipid content and fatty-acid composition

In the three stranded bottlenose whales, blubber lipids were dominated by wax esters throughout the blubber layer (Table 5). Triglycerides occurred in small amounts in the middle layer and in slightly higher amounts in the outer layer. Fatty-acid composition also varied between the inner, middle, and outer layers of blubber; however, the magnitude of differences was not generally large (Fig. 1a). In particular, the overall pattern of fatty acids was similar between layers in that the most abundant fatty acids in the inner and middle layers were also the most abundant in the outer layer and the least abundant in the inner and middle layers were also the least abundant in the outer layer (e.g., Fig. 1a). The small sample sizes (three animals and three blubber locations) precluded powerful statistical comparison of sites.

Table 2. Results of analyses of the stomach contents of two northern bottlenose whales stranded in eastern Canada.

Squid species	Bottlenose whale code	
	Ha-01–92	Ha-97–01
<i>Gonatus steenstrupi</i>	720 (55)	1358 (87.2)
<i>Taonius pavo</i>	328 (25)	75 (4.8)
<i>Teuthowenia</i> sp.	223 (17)	—
<i>Histioteuthis reversa</i>	—	116 (7.5)
<i>Histioteuthis dofleini</i>	—	5 (0.3)
<i>Histioteuthis heteropsis</i>	—	1 (0.1)
<i>Histioteuthis</i> sp.	11 (1)	—
<i>Chiroteuthis</i> spp.	20 (1.5)	—
<i>Alloposus mollis</i>	7 (0.5)	2 (0.1)
Total		
Lower beaks	1309	1557
Upper beaks	2533	3474

Note: The number of lower beaks of each species is shown; numbers in parentheses show the percentage that this represents.

The fatty-acid composition of the blubber biopsy samples taken from live animals was in general similar to that observed in all blubber layers of stranded animals (Figs. 1a and 1b). The unusual short-chained fatty acid isovaleric acid, found in some odontocete species (e.g., Koopman et al. 1996), was not detected in bottlenose whale blubber. The proportions of major fatty acids contained in blubber biopsy samples were significantly different from those of the inner and middle layers, but not from those of the outer layer, of stranded animals (MANOVA, biopsy samples vs. inner layer, $F = 22.1$, $p < 0.001$; biopsy samples vs. middle layer, $F = 6.8$, $p = 0.004$; biopsies vs. outer layer, $F = 1.9$, $p = 0.182$). However, again the patterns were generally similar, suggesting that biopsy samples (which contain only the outer blubber layer) may be used to infer some qualitative aspects of diet in this species.

There was no significant difference in proportions of major fatty acids between male and female bottlenose whales (MANOVA, $p = 0.321$; Fig. 1). However, k -means clustering into two groups based on major fatty acids divided the biopsy samples into groups according to sex with 87.5% success: a group of 5 animals (4 of which were male) and a group of 12 animals (11 of which were female). It appears, therefore, that there may be underlying dietary differences between males and females, but that the sample sizes are too small to allow these differences to be detected statistically.

The fat content of adult *G. fabricii* was higher ($8.3 \pm 1.52\%$) than that of juvenile *G. fabricii* ($4.6 \pm 0.61\%$), although small sample sizes prohibited detection of a statistical difference (t test, $p = 0.118$; Table 4). Juveniles and adults also showed large differences in fatty-acid composition. Juvenile *G. fabricii* contained high levels of the dietary fatty acids 20:5n–3 and 22:6n–3, while adult *G. fabricii* contained low levels of these but high levels of 20:1n–9 and 22:1n–11 (Fig. 1c). Fatty-acid patterns in biopsy samples from bottlenose whales exhibited some characteristics similar to those of adult *G. fabricii*, with both containing relatively high levels of 18:1n–9, 20:1n–9, and 22:1n–11 concurrent with low levels of 16:0, 20:5n–3, and 22:6n–3. These were particularly different from those found in juvenile *G. fabricii*,

Table 3. Biopsy samples from northern bottlenose whales in the Gully.

Sample No.	Date collected	ID No.	Year ID No. first seen	Sex	Stable-isotope analysis (‰)		Fatty-acid analysis
					$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	
3	16 July	1289	1990	F	-17.77	14.73	√
4	16 July	—	—	F	-17.45	15.19	√
5	16 July	—	—	F	-16.65	15.32	√
6	16 July	54	1988	F	-17.34	15.32	√
8	12 Aug.	1000	1996	F	-17.15	15.12	√
9	12 Aug.	1313	1997	F	-17.58	15.44	√
10	12 Aug.	—	—	F	-17.36	15.03	—
11	13 Aug.	1318	1997	F	-17.59	15.30	√
12	13 Aug.	1315	1997	F	-17.24	15.04	√
13	13 Aug.	619	1993	F	-17.19	14.39	√
14	14 Aug.	—	—	F	-16.68	15.32	√
15	14 Aug.	—	—	M*	-17.29	15.59	√
16	14 Aug.	480	1990	M*	-17.74	15.59	√
17	16 Aug.	1039	1996	M*	-17.39	15.50	√
18	16 Aug.	—	—	M	-17.06	15.51	√
19	16 Aug.	1336	1997	F	-18.09	15.50	√
20	16 Aug.	—	—	M	-17.86	15.39	√
Mean \pm 1 SEM					-17.38 \pm 0.09	15.25 \pm 0.08	

Note: Details of individual whales, stable-isotope values, and whether samples were processed for fatty-acid composition are shown.

*Field or photographic identification as mature.

Table 4. *Gonatus fabricii* samples from the Norwegian Sea.

Station	ID No.	Age and sex	Mantle length (cm)	Body mass (g)	Stable-isotope analysis (‰)		Lipid content (%)	Fatty-acid analysis
					$\delta^{13}\text{C}$	$\delta^{15}\text{N}$		
276	1218-1	Adult female	26.0	179.12	-19.60	13.60	6.34	√
	1216-2	Adult female	30.2	214.03	-19.22	14.23	7.26	√
280	0112-5	Adult male	21.5	83.28	-19.55	13.40	11.28	√
	0112-6	Juvenile	—	3.94	—	—	5.73	√
	0112-7	Juvenile	—	3.65	—	—	5.49	√
	0112-8	Juvenile	—	na	—	—	3.40	√
	0112-9	Juvenile	—	2.68	—	—	3.60	√
	0112-10	Juvenile	—	na	-19.92	9.73	—	—
	0112-11	Juvenile	—	na	-19.59	9.22	—	—

Note: Collection details (see Bjørke et al. 1997), stable-isotope values, lipid contents, and whether samples were processed for fatty-acid composition are shown; na, not available.

Table 5. Lipid-class composition of bottlenose whale blubber ($n = 3$ stranded animals).

Blubber layer	Wax esters (%)	Triglycerides (%)	Free fatty alcohol (%)	Phospholipid (%)
Inner	96.02 \pm 0.62	0.00	3.77 \pm 0.62	0.21 \pm 0.21
Middle	92.70 \pm 5.63	6.13 \pm 6.13	1.00 \pm 1.00	0.18 \pm 0.18
Outer	85.82 \pm 3.48	14.18 \pm 3.48	0.00	0.00

Note: Values are given as the mean \pm SEM.

as well as herring and redfish, all of which contained quite high levels of 16:0, 20:5 n -3, and 22:6 n -3 (Fig. 1). A plot of all samples for the first two principal components of transformed major fatty acids shows the relative similarity of fatty acids in the inner, middle, and outer layers of bottle-

nose whale blubber to one another. Furthermore, this plot shows that the fatty acids in bottlenose whale blubber were more similar to those in adult *G. fabricii* than to those in juvenile *G. fabricii*, herring, or redfish (Fig. 3). The fatty acids of bottlenose whales were also more similar to those of the

Fig. 1. Selected major fatty acids (mass percent) in the inner, middle, and outer blubber layers of stranded northern bottlenose whales (*Hyperoodon ampullatus*) sampled at a midlateral site below the dorsal fin (a), in biopsy samples of the blubber of free-ranging male and female bottlenose whales (b), in adult and juvenile squid (*Gonatus fabricii*) (c), and in herring and redfish caught on the Scotian Shelf, eastern Canada (S.J. Iverson, W.D. Bowen and R.G. Ackman, unpublished data) (d). Bars are means and vertical lines are 1 SEM. (The full dataset for fatty-acid composition of bottlenose whale and *G. fabricii* samples is presented in Hooker 1999.)

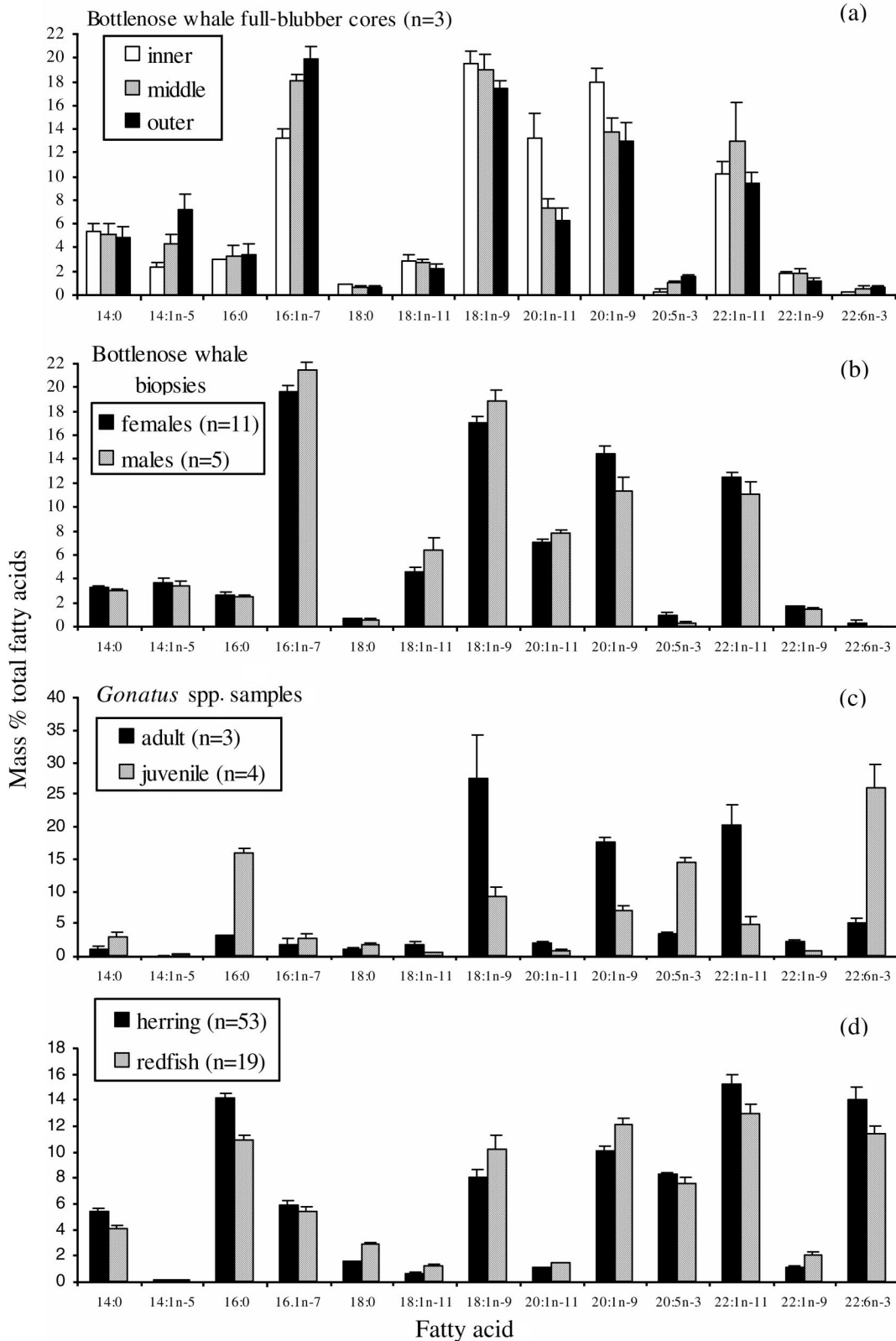


Fig. 2. Stable carbon and nitrogen isotope ratios of skin samples from bottlenose whales in the Gully and of *G. fabricii* samples collected in the Norwegian Sea. Bottlenose whale samples are shown separately for males and females. *Gonatus fabricii* samples are shown separately for adults and juveniles. (Each point represents the mean and lines show 1 SEM.)

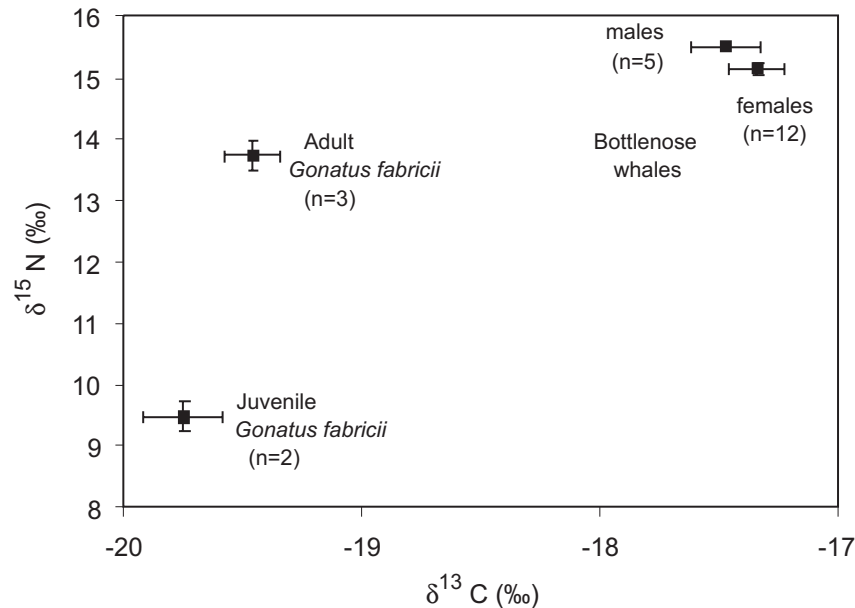
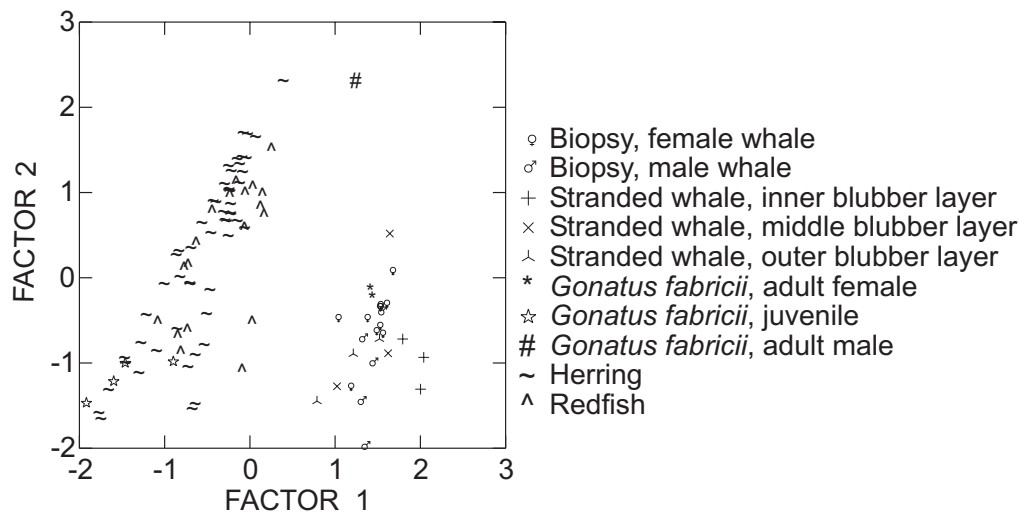


Fig. 3. Principal component analysis using major fatty acids for all samples. Factor 1 explains 54% of variance and factor 2 explains 19% of variance.



two adult female *G. fabricii* samples than to the adult male *G. fabricii* sample. However, since only one adult male squid was sampled, conclusions are quite limited (Fig. 3).

Stable isotopes

Bottlenose whale skin samples had a small range of stable-isotope values, with nitrogen-isotope values ranging from 14.39 to 15.59‰ ($15.25 \pm 0.08\text{‰}$ (mean \pm SD), $n = 17$) and carbon-isotope values ranging from -18.09 to -16.65‰ ($-17.38 \pm 0.09\text{‰}$, $n = 17$; Table 3, Fig. 2). Male bottlenose whales had somewhat higher levels of isotopic nitrogen than females (males: $15.52 \pm 0.04\text{‰}$ $\delta^{15}\text{N}$, $n = 5$; females: $15.14 \pm 0.09\text{‰}$ $\delta^{15}\text{N}$, $n = 12$; Fig. 2). This difference was significant (t test, $p = 0.002$), but was only twice the magnitude of the reproducibility of the analysis (0.2‰). However, those indi-

viduals identified as mature males (Table 3) had the highest nitrogen-isotope values (15.50 – 15.59‰ for adult males compared with 14.73 – 15.50‰ for other whales), which suggests that this difference may have biological relevance. There was no significant difference between levels of isotopic carbon between males and females (t test, $p = 0.514$; males: $-17.47 \pm 0.33\text{‰}$ $\delta^{13}\text{C}$, $n = 5$; females: $-17.34 \pm 0.41\text{‰}$ $\delta^{13}\text{C}$, $n = 12$; Fig. 2).

The nitrogen-isotope values for adult *G. fabricii* were significantly different from those for juvenile *G. fabricii* (t test, $p = 0.002$; adults: $13.74 \pm 0.25\text{‰}$ $\delta^{15}\text{N}$, $n = 3$; juveniles: $9.47 \pm 0.25\text{‰}$ $\delta^{15}\text{N}$, $n = 2$; Fig. 1, Table 4). However, the carbon-isotope values for adult *G. fabricii* did not differ significantly from those for juvenile *G. fabricii* (t test, $p = 0.285$; Fig. 2).

Discussion

We used three techniques, stomach-content, fatty-acid, and stable-isotope analyses, to make inferences about the diet of northern bottlenose whales in the Gully. Singly, each of these techniques is only suggestive, but taken together they corroborate the likely diet of this species in the region, and thus provide the foundation needed for ecosystem management in this recently designated marine protected area.

The results from stomach-content analyses of bottlenose whales stranded in eastern Canada are similar to those from stomach-content analyses of these whales elsewhere in the North Atlantic, suggesting that one of their primary prey is a squid of the genus *Gonatus*, either *G. fabricii* or *G. steenstrupi*. Both of these species are found across the North Atlantic, and the two were only recently separated (Kristensen 1981). In fact, many previous observations of *G. fabricii* in the diet of bottlenose whales were made prior to the identification of *G. steenstrupi*. The two species appear to be largely differentiated by latitude, with *G. fabricii* having a largely arctic distribution, while *G. steenstrupi* has a more boreal distribution to the south of that of *G. fabricii*. Unfortunately, *Gonatus* that have been trawled near the Gully were not differentiated to species (Dawe and Stephen 1988). However, based on the distribution of *Gonatus* sampled from other locations, it appears that bottlenose whales in the location of the Gully are more likely to be feeding on *G. steenstrupi* than on *G. fabricii*.

Gonatus fabricii samples

Differences in the fatty-acid and stable-isotope composition of the *G. fabricii* samples appear to reflect what is known of the life history of these squid in terms of the differences in foraging ecology between adults and juveniles. Furthermore, results suggest that they represent a relatively rich food source. It is generally thought that squid are relatively low in fat content compared with marine fish (Sidwell et al. 1974). However, samples of both adult and juvenile *G. fabricii* were found to have relatively high lipid contents, with a higher lipid content in adults than in juveniles (Table 4). The values observed here, ranging from 3.4 to 11.3% lipid, are much higher than those found for other squid species (e.g., 2% lipid in the short-finned squid, *Illex illecebrosus*; Kirsch et al. 1998). The adult females had a much lower fat content than the adult male, possibly because of recent spawning (both females possessed spermatophores; Bjørke et al. 1997). A calorific value of 3.78 kJ/g wet mass has previously been determined for some small *G. steenstrupi* (Clarke et al. 1985), although it was noted that the calorific value of adults would be higher. Clarke et al. (1985) suggested that it is the low-density oil in their livers (used for buoyancy) that elevate the calorific values of the Gonatidae.

The fatty-acid composition of adult *G. fabricii* was distinctly different from that of juveniles (Fig. 1c). Juvenile *G. fabricii* contained relatively high quantities of the fatty acids 20:5n-3 and 22:6n-3, whereas adults contained little of these but relatively high quantities of 20:1n-9 and 22:1n-11. A difference between adult and juvenile squid was also reflected in their $\delta^{15}\text{N}$ composition. Adult *G. fabricii* contained much more $\delta^{15}\text{N}$ ($13.7 \pm 0.25\text{‰}$, $n = 3$) than juveniles ($9.47 \pm 0.25\text{‰}$, $n = 2$). Nitrogen-isotope values are useful for making inferences about trophic level in the marine environment. An increase of approximately 3‰ in $\delta^{15}\text{N}$ is

generally thought to reflect a trophic-level increase (Minagawa and Wada 1984). The enrichment of 4.2‰ in ^{15}N therefore suggests that adult *G. fabricii* are approximately 1.2–1.4 trophic levels higher than juveniles.

Such a suggestion of dietary differences between adults and juveniles based on nitrogen-isotope values is consistent with previous assessments of their diet. North Atlantic *Gonatus* spp. are thought to have distinct ecological requirements at different life stages, with juveniles (up to 3 cm pen length) living in the surface waters (to 80 m depth) and later showing an ontogenetic descent to the sea floor. Juveniles prey on various zooplankton, primarily copepods and euphausiids, but also amphipods, pteropods, and chaetognaths (Nesis 1965; Kristensen 1983). In contrast, copepods and euphausiids have never been recorded as prey of adult *Gonatus* spp. The major prey of adults instead are mainly amphipods, isopods, and decapods (shrimps and mysids). At all ages, *Gonatus* spp. will take the largest prey possible; for adults this includes fish and other squid. Cannibalism is also known (Kristensen 1983, 1984), which would further raise the apparent trophic level of cannibalistic individuals (Hobson and Welch 1995).

Previous studies of the fatty-acid composition of squid have focused on shallow-water species. Short-finned squid from the North Atlantic were found to be high in the dietary fatty acids 20:5n-3 and 22:6n-3 but low in the fatty acids 20:1n-9 and 22:1n-11 (Kirsch et al. 1998). A similar pattern (high in 20:5n-3 and 22:6n-3) was also noted for unspecified small squid in Prince William Sound, Alaska (Iverson et al. 1997). This pattern was also found for juvenile but not adult *G. fabricii* (Fig. 1). The extremely low levels of 20:5n-3 and 22:6n-3 in adult *G. fabricii* are relatively unusual in marine prey species (S.J. Iverson, personal observation), making their comparison with the similar fatty-acid patterns found in bottlenose whales intriguing.

Relative to the nitrogen-isotope value for our adult *G. fabricii* samples ($13.7 \pm 0.3\text{‰}$), values for other squid species in the North Atlantic have tended to be low: $11.9 \pm 0.1\text{‰}$ $\delta^{15}\text{N}$ for short-finned squid (Hobson and Montevecchi 1991) and $12.2 \pm 0.2\text{‰}$ $\delta^{15}\text{N}$ for the long-finned squid, *Loligo pealei* (Abend and Smith 1997). Most other published data on squid from the Pacific Ocean also tend to show low $\delta^{15}\text{N}$ values ($11.7 \pm 0.4\text{‰}$ for neon flying squid, *Ommastrephes bartrami*, Gould et al. 1997; $9.6 \pm 0.5\text{‰}$ for unspecified small squid, Hobson et al. 1997; 10.95‰ for *Taonius pavo* and 8.7 and 11.8‰ for *Histioteuthis dofleini*, P. Ostrom, unpublished data). However, as in our study, a single large gonatid squid from the North Pacific was found to contain 16.7‰ $\delta^{15}\text{N}$ (Hobson et al. 1997). If high nitrogen-isotope values for large gonatid individuals are not related to ^{15}N enrichments at the base of their food web, the isotope data suggest that *Gonatus* species are positioned at a higher trophic level than other squid species.

Lipids in bottlenose whale blubber

The blubber of bottlenose whales showed some unusual characteristics compared with that of many other odontocete species. The high proportion of wax esters found in bottlenose whale blubber (Table 5) has been previously noted in this and other beaked whales: samples of Baird's beaked whale (*Berardius bairdii*), dense-beaked whale (*Mesoplodon*

densirostris), and northern bottlenose whale contained 98, 99, and 94% wax esters, respectively (Litchfield et al. 1976). The Physeteridae also have a high wax-ester content, with sperm whales recorded at 60–85% and dwarf sperm whale (*Kogia sima*) at 42% (Litchfield et al. 1976; Lockyer 1991). The high prevalence of wax esters among the Ziphiidae and Physteridae appears to be related to phylogeny, but its function is largely unknown (e.g., Koopman et al. 1999; Koopman 2001).

In animals that store lipid as wax esters (a long-chain fatty alcohol esterified to a fatty acid), the fatty alcohols arise from the conversion of a fatty acid to the corresponding chain-length fatty alcohol, in a process that may be selective for specific fatty acids (Bandi and Mangold 1973; Sargent et al. 1976; Lee and Patton 1989). However, typical fatty-acid analyses, as described here, methylate and analyse only the fatty acids released from triglycerides and wax esters. Since the fatty alcohols in the wax esters are not analysed, this may potentially bias interpretations of the original fatty-acid pool with respect to diet interpretation. The available biopsy samples contained insufficient material for reanalysis, but several of the blubber samples from stranded whales were reanalysed to include a conversion of these alcohols to fatty acid methyl esters (S. Budge and S.J. Iverson, unpublished data). While the results suggest some differences in individual fatty acids, the general fatty-acid signature remained similar, especially in that the most abundant and least abundant fatty acids are the same under both analyses. Thus, since we are using our data for qualitative inferences only, our conclusions remain the same based on either analysis.

The blubber of bottlenose whales in the western North Atlantic appears to contain primarily (82–88%) mono-unsaturated fatty acids (details are provided in Hooker 1999). The major (most abundant) fatty acids found in bottlenose whales are illustrated in Fig. 1. Of these, several can be produced endogenously through biosynthesis, including 16:0, 18:0, and especially 14:1*n*-5, 16:1*n*-7, and 18:1*n*-9 (Kirsch et al. 2000), although all but 14:1*n*-5 are also likely to come significantly from the diet. Thus, their levels could reflect biosynthesis in addition to prey consumed (Iverson 1993; Kirsch et al. 2000). However, the major fatty acids, such as 20:1*n*-11, 20:1*n*-9, 20:5*n*-3, 22:1*n*-11, and 22:6*n*-3, are likely to be primarily dietary in origin and therefore most reflective of prey consumption (Iverson 1993). The isomers of the long-chain monounsaturates (22:1 and 20:1) are believed to originate from the same-chain-length fatty alcohols of wax esters of some copepods, which are converted to fatty acids during digestion and carried up marine food chains (Ackman and Eaton 1966; Pascal and Ackman 1976; Ackman 1980; Ackman et al. 1980; Iverson 1993; Iverson et al. 1997).

Possibility of using fatty acids from blubber biopsy samples to infer aspects of diet

The blubber of most cetaceans studied appears to show marked stratification in fatty-acid composition across its depth, from that nearest the body core to that nearest the skin (e.g., balaenopterid whales, Ackman et al. 1975; Lockyer et al. 1984; sperm whales, Lockyer 1991; harbour porpoises, *Phocoena phocoena*, and other odontocetes, Koopman et al. 1996; Koopman 2001). Among species studied, longer chain unsaturated fatty acids, typical of diet, are more prevalent in

the inner blubber layer, with typically biosynthesized fatty acids more prevalent in the outer layer, suggesting that the inner layer is more metabolically active in terms of fat storage and lipid deposition from the diet. Northern bottlenose whales also exhibited differences in lipid-class and fatty-acid composition across the blubber layer (Table 5, Fig. 1a). However, this stratification was much less pronounced than has been found in smaller cold-water cetaceans such as harbour porpoises and belugas (*Delphinapterus leucas*; Koopman et al. 1996; S.J. Iverson, personal observation). For instance, in the harbour porpoise, when important fatty acids of dietary origin such as 20:1*n*-9, 22:1*n*-11, 20:5*n*-3, and 22:6*n*-3 occur in the inner layer at levels of about 8.8, 10.0, 4.0, and 7.5%, respectively, they are found in the outer layer at 1.2, 1.2, 0.7, and 0.4%, respectively (Koopman et al. 1996). These represent differences (inner to outer) of 7.3-, 8.3-, 5.7-, and 18.8-fold, respectively. In comparison, the differences in the same fatty acids across the inner and outer blubber layer in the three stranded bottlenose whales were 1.4-, 1.1-, 0.2-, and 0.3-fold, respectively. Thus, differences across blubber depth in bottlenose whales do occur and preclude the use of outer-layer biopsies for quantitative evaluation of diet. However, the relative similarity in fatty-acid patterns of inner and outer blubber layers in the stranded animals, coupled with their similarity to those of the biopsy samples (Fig. 1), suggests that biopsy samples can be used to investigate at least some qualitative or relative aspects of diet for this species.

Potential dietary information from bottlenose whale biopsy samples

Although we cannot compare the bottlenose whale biopsy samples with gonatid samples from the Gully, gonatid samples from the Norwegian Sea may serve as a general index of the underlying fatty-acid signature for deep-sea *Gonatus* species in the North Atlantic. Elsewhere, within-species differences in fatty-acid composition have been observed among locations; however, individuals could still be identified to species as a whole (Iverson et al. 1997). Likewise, regional differences between the eastern and western North Atlantic likely exist, but this could not be assessed, owing to the difficulty of acquiring samples of these deep-sea squid from canyon areas such as the Gully.

The fatty-acid composition of the bottlenose whale biopsy samples, with a high percentage of 20:1*n*-9 and 22:1*n*-11 and a low percentage of 20:5*n*-3 and 22:6*n*-3, was more similar to that of the adult *G. fabricii* than to that of the juvenile *G. fabricii* or the herring and redfish samples (Figs. 1 and 3). Although herring were also high in 20:1*n*-9 and 22:1*n*-11, this was concurrent with high levels of 20:5*n*-3 and 22:6*n*-3. The overall fatty-acid compositions of these species were therefore more different from those of the biopsied bottlenose whales than was the composition of adult *G. fabricii* (Figs. 1 and 3), suggesting that these are less likely to be major prey of bottlenose whales in the Gully. Similarly, no other prey species have yet been recorded with a fatty-acid signature similar to that of bottlenose whales (e.g., with high 20:1 and 22:1 isomers but low 20:5*n*-3 and 22:6*n*-3; Dahl et al. 2000; S.J. Iverson, personal observation). While this does not confirm that gonatids are a major prey item of bottlenose whales in the Gully, their fatty-acid

Fig. 4. Stable-isotope values for North Atlantic odontocetes. Sample sizes are shown in parentheses. Sampling locations and sources are as follows: ^amuscle samples, Arctic Ocean (Hobson and Welch 1992); ^bmuscle samples, Newfoundland (Ostrom et al. 1993); ^cskin samples, the Gully (this study); ^dtooth samples, Atlantic U.S.A. (Walker and Macko 1999); ^eskin samples, North Atlantic Ocean (Abend and Smith).

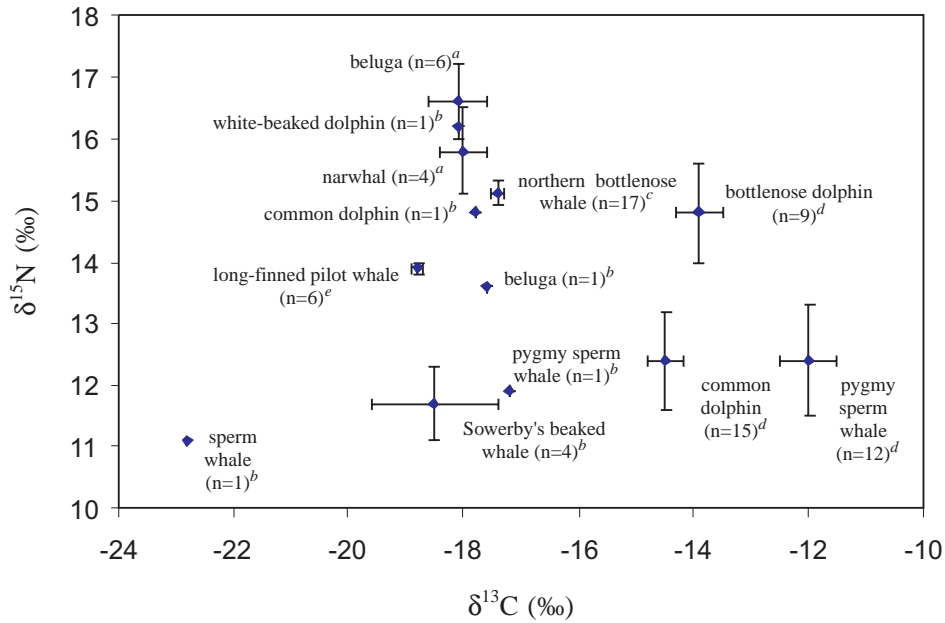


Table 6. Nitrogen-isotope values (mean ± SEM ‰) for similar trophic levels recorded in eastern Canada and the North Pacific Ocean.

	Norway	Eastern Canada	North Pacific	Difference
Adult/large <i>Gonatus</i> spp.	13.7 ± 0.3 ^a		16.7 ^b	
Herring (<i>Clupea</i> sp.)		11.2 ^c	14.5 ± 0.1 ^b	3.3
Euphausiids		7.9 ± 0.2 ^c	11.2 ± 0.5 ^b	3.3

^aThis study (Norwegian Sea).
^bHobson et al. 1997 (Gulf of Alaska and North Pacific).
^cFry 1988 (Georges Bank, eastern Canada).

signatures are at least consistent with such a possibility. Furthermore, although it is only anecdotal, the fatty-acid composition of bottlenose whales was more similar to that of adult female *G. fabricii* samples than to that of the adult male *G. fabricii* sample (Fig. 3). Adult female gonatids are known to become relatively immobile during their bathypelagic mating and spawning stage, whereas adult males remain active swimmers, which has led to previous suggestions that these nonactive adult females would be the preferred prey of *Gonatus*-eating whales and dolphins (Arkhipkin and Bjorke 1999).

Bottlenose whales had generally high nitrogen-isotope values (15.3 ± 0.1‰ δ¹⁵N) relative to published results for other odontocete species in the North Atlantic (Fig. 4). Although several considerations need to be factored into such a comparison (e.g., variation in location and thus potential differences at the base of the foodweb (Dunton et al. 1989) or differences between tissue types sampled (Tieszen et al. 1983; Abend and Smith 1997)), these are comparatively minor. Making inferences based on δ¹³C values is more difficult, since trophic enrichment is much less than for δ¹⁵N. Furthermore, other factors come into play: δ¹³C values are typically

higher in coastal or benthic food webs than in pelagic food webs (McConnaughey and McRoy 1979; Dunton et al. 1989), and higher at lower latitudes than at higher latitudes (Burton and Koch 1999; see Fig. 4).

Results from stable-isotope analyses are less definitive than those from fatty-acid analyses in terms of suggesting a diet based largely on gonatids, but they are largely consistent with such a hypothesis. The nitrogen-isotope value provided for a single sample of redfish from eastern Canada (14.2‰; Hobson and Montevecchi 1991) suggests that redfish cannot make up a large component of the bottlenose whale diet in the Gully (since 3‰ enrichment between dietary items and their consumer is expected). Likewise, although a single sample of herring (11.2‰; Fry 1988) suggests that this could be consistent with the bottlenose whale diet, the fatty-acid values counter this. Comparing stable-isotope values between bottlenose whales and *G. fabricii* samples is problematic, since the two datasets were collected from regions on either side of the North Atlantic. However, we can estimate the isotopic composition of *G. steenstrupi* from the Gully through a comparison of isotope values from different ecosystems (Table 6). Interpolating the nitrogen-isotope value for gonatids in the

Gully region based on the isotope value for a gonatid from the North Pacific we obtain a value of 13.4‰, which is 0.3‰ lower than that measured from the Norwegian Sea samples. The estimate differs by 1.9‰ from the nitrogen-isotope value for the bottlenose whale, which is consistent with a diet consisting of a large portion of adult gonatids supplemented with lower trophic level organisms.

Male and female bottlenose whales showed significant differences in $\delta^{15}\text{N}$ level and there appeared to be some differences in their fatty-acid composition, such that *k*-means clustering based on major fatty acids identified the two groupings with 87.5% accuracy (Figs. 1 and 2). Two of the biopsied animals (Nos. 15 and 16) were identified in the field as large mature males, and another (No. 17) was identified photographically as a mature male, and the samples from these animals also had the highest nitrogen-isotope values (15.59‰ for the first two and 15.50‰ for the third). While anecdotal, this is suggestive of dietary differences between adult males and other bottlenose whales in the Gully. In previous studies, differences in nitrogen-isotope values have also been recorded as a function of age and (or) sex. For example, adult male Steller sea lions (*Eumetopias jubatus*) show a higher trophic position than adult females, and adult females a higher trophic position than juvenile males (Hobson et al. 1997). These authors suggested this was likely due to differential reliance on certain food items or a difference in the size of prey taken. Similarly, the dietary difference observed between mature male and female bottlenose whales could be due to differences in prey size, class, or composition.

In conclusion, this study demonstrates the potential utility of remote biopsy samples for making dietary inferences in northern bottlenose whales. Nevertheless, we stress that it will be essential to determine the degree and influence of fatty-acid stratification in the blubber of any species under study before extending this concept to other cetaceans. In bottlenose whales, however, the three different techniques investigated here, fatty-acid and stable-isotope analyses of skin and blubber biopsy samples and analysis of the stomach-contents of stranded animals, all give complementary results. While none conclusively demonstrate that squid of the genus *Gonatus* are the major items in the diet of northern bottlenose whales in the Gully, the general agreement between the results of all three techniques supports this hypothesis. Furthermore, the results suggest selective feeding on adult rather than juvenile gonatids (Figs. 1 and 2). This is consistent with the known depth distribution of adult North Atlantic gonatids and the foraging ecology of bottlenose whales (Kristensen 1984; Bjørke et al. 1997; Hooker and Baird 1999). Although the *G. fabricii* samples investigated were from an ecosystem different from that of the Gully, the unique pattern of fatty acids found in the blubber of bottlenose whales from the Gully is not consistent with that of other recorded prey species. Further qualitative resolution of the diet of bottlenose whales in the Gully may be achieved by fatty-acid and stable-isotope analysis of gonatid specimens from the western North Atlantic, as well as other deep-water prey from the Gully region. Quantitative evaluation of diet based on fatty-acid composition would require deeper biopsies that sample the innermost blubber layer.

Acknowledgments

Many thanks are extended to all crew members of *Balaena*, particularly R.W. Baird and B. Carter, for assistance during collection of biopsy samples. Fieldwork and laboratory analyses were funded by operating and equipment grants (to H. Whitehead and S.J.I.) from the Natural Sciences and Engineering Research Council of Canada, the Canadian Federation of Humane Societies, and World Wildlife Fund, Canada. The Nova Scotia Stranding Network (Halifax, N.S.), the Canadian Cooperative Wildlife Health Centre (College of Veterinary Medicine, University of Montreal, Montreal, Que.), and L. Measures at the Department of Fisheries and Oceans (Mont-Joli, Que.) provided blubber samples from stranded animals. The Nova Scotia Stranding Network and L. Measures also provided stomach contents from these stranded bottlenose whales. H. Bjørke at the Institute of Marine Research, Bergen, Norway, provided samples of *G. fabricii* for comparative analyses. H.N. Koopman generously analysed the stranded bottlenose whale samples for lipid-class composition and provided very useful insight on ideas of wax ester metabolism and synthesis. We also thank S. Budge for analysing wax ester fatty alcohols in blubber. R.W. Baird, H. Bjørke, H.N. Koopman, and H. Whitehead provided useful comments on previous versions of the manuscript.

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