LIPID METABOLISM BY RIGHT WHALES: USING FECAL SAMPLES TO ASSESS ASSIMILATION OF COPEPOD TRIACYLGLYCEROLS AND WAX ESTERS

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ABSTRACT

In mammals, reproductive health and success are closely linked to nutritional health and body condition, particularly for females. Thus, adequate and reliable food resources are required for populations to remain stable, or to increase. In the Bay of Fundy, right whales (*Eubalaena glacialis*) feed almost exclusively on stage V copepods, *Calanus finmarchicus* (C5). *Calanus* possesses two classes of storage lipids, triacylglycerols (TAG) and wax esters (WE), the latter making up 94% of the total lipid present. Interestingly, most mammals are incapable of metabolizing WE and consequently eliminate WE in their feces. Current energetic models assume that right whales are utilizing all of the lipids from their copepod prey. This study aims to determine whether right whales can metabolize all copepod lipids by comparing estimates of the lipids a right whale consumes with those that are eliminated in the feces. Using data from 60 copepod and 24 fecal samples, a right whale ingestion model and known copepod lipid composition, I estimated that an average right whale (40000 kg) ingests approximately 62000 g of lipid per day. The majority of total lipid ingested (~ 58000 g) consists of WE. Using allometrically-derived estimates of defecation rates in conjunction with the lipid composition of fecal material, I calculated that an average right whale eliminates approximately 250 g of WE per day, implying that right whales are assimilating over 99 % (57765 g) of the ingested WE. The composition of fecal material differed significantly from that of the diet, suggesting that eliminated lipids might originate from a source other than diet. Copepod lipid composition was dominated by long chain saturated fatty acid (FA) components 14:0 (14.73 ± 0.32 wt%) and 16:0 (8.99 ± 0.20 wt%), with monounsaturated and polyunsaturated components comprising the

remainder of the FA composition. Long chain monounsaturated 20:1n-9 and 22:1n-11 dominated the fatty alcohol (FAlc) composition. FAlc components comprised only a small portion (< 3.0 %) of right whale fecal material, suggesting preferential or perhaps complete metabolism of certain FA and FAlc components. Fecal lipid composition consisted primarily of saturated FA components, many of which were absent in copepod lipid composition. These data suggest that right whales have evolved an unusual metabolic mechanism, such as specialized enzymatic machinery or a gut symbiont, which unlike other animals enables them to utilize most of their WE-rich diet.

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INTRODUCTION

Prey selection is related to the digestive capabilities of the predator (McKey et al. 1981). Therefore, the food selected by an animal will be correlated to the digestibility of specific nutrients contained in that food (McNab 2002). Many organisms rely on specific food resources to meet energetic requirements, however the digestive mechanisms used to take advantage of different food types vary among species. In vertebrates, animals are either generalists, making use of a variety of food resources, or are highly specialized. For example, koalas (*Phascolarctos cinereus*) are extreme specialists that feed only on a few species of *Eucalyptus* (McNab 2002). In the marine environment, several turtle species, such as leatherbacks (*Dermochelys*), which feed primarily on jellyfish and tunicates, and green turtles (*Chelonia*), which feed on seagrasses and algae (Mortimer 1982), are specialized foragers. Honeyguides (Indictoridae) are small birds that seek out beeswax (wax is thought to be indigestible in birds and mammals) and ignore bee larvae and honey stored in the beehive. Western North Atlantic right whales (*Eubalaena glacialis*) provide another interesting example of predator specialization by foraging almost exclusively on a prey item whose main lipid components (wax esters) are metabolically unavailable to most mammalian species (Hansen & Mead 1965, Place 1992a). To date however, the capacity for right whales to utilize all of the nutrients in their prey has not been investigated. This study aims to determine the metabolic capabilities of right whales, specifically, the Western North Atlantic population, by measuring the digestibility of specific lipid components that are found in their primary prey, the calanoid copepod, *Calanus finmarchicus*.

Reproductive success and population growth in mammalian species are dependent largely on foraging success, therefore prey quality and availability are critical to a species' overall success and fitness. In mammals, reproductive health and success are closely linked to nutrition and body condition, particularly for the female (Lockyer 1981a, Stirling et al. 1999). Thus, adequate and reliable food resources are essential for populations to remain stable or to increase. Calanoid copepods represent an important food resource for many fish, pelagic seabirds and mammals in the Bay of Fundy, including the North Atlantic right whale (*Eubalaena glacialis*). North Atlantic right whales are one of the most endangered species of large whales (Clapham et al. 1999) with approximately 350 individuals remaining in the population (Kraus et al. 2001). Right whale populations have suffered severe declines due to over-exploitation from whaling and more recently, anthropogenic mortality from ship strikes (Moore et al. 2007) and fishing-gear entanglement (Johnson et al. 2007). There is also evidence of decreased reproductive rates in this population, particularly in the late 1990s (Knowlton & Kraus 2001, Kraus et al. 2001, Kraus et al. 2007). The specific cause of this reproductive variability remains unknown, however several factors have been suggested, including exposure to contaminants or marine biotoxins, disease, reduced genetic diversity and poor body condition (Reeves et al. 2001, Rolland et al. 2007).

It has also been suggested that the lack of recovery of North Atlantic right whale populations is due to the fact that extensive whaling pressure allowed other copepod predators (*i.e.* sand lance, herring and basking sharks) to flourish (Payne et al. 1990), therefore leaving insufficient prey quantities for North Atlantic right whales (IWC 2001). Population models suggest that right whales will go extinct within the next two centuries

if current trends in mortality and reproduction continue (Caswell et al. 1999, Fujiwara & Caswell 2001).

Baumgartner & Mate (2003) and Michaud & Taggart (2007) have suggested that prey quality and availability may be contributing to low reproductive rates in North Atlantic right whales. Although adequate and reliable food resources are recognized as important ecological factors associated with population success and fitness, the ability to efficiently utilize the available energy from the diet is an important requirement that is less often addressed. For example, red pandas (*Ailurus fulgens*) digest proteins and fat from bamboo shoots more efficiently than from bamboo leaves (Wei et al. 1999).

Right whales preferentially feed on older, stage V (C5) copepods (*C. finmarchicus*) in the Bay of Fundy (Murison & Gaskin 1989, Baumgartner & Mate 2003). In the lower Bay of Fundy, C5 copepods are most abundant during the mid to late summer months when right whales are also present in their greatest numbers (Murison & Gaskin 1989, Woodley & Gaskin 1996, Baumgartner et al. 2003a, Michaud & Taggart 2007). Murison & Gaskin (1989) and Baumgartner & Mate (2003) showed that C5 *C. finmarchicus* are the dominant zooplankton near feeding right whales and inhabit depths below 100 m. Right whales rely on dense patches of C5 *C. finmarchicus* to efficiently exploit these deep-water aggregations (Baumgartner et al. 2007). C5 copepods are thought to be an important prey item for right whales because they contain the highest caloric content of any copepod life phase, an adaptation to facilitate over wintering diapause (Comita et al. 1966).

Calanus copepods are composed of several lipid classes (Michaud & Taggart 2007), including two types of storage lipids, triacylglycerols (TAG) and wax esters (WE).

In most animals, TAG are rapidly metabolized and are more efficiently hydrolyzed than WE, and thus TAG are the primary form of ingested and stored lipids in most mammals (Savory 1971, Patton & Benson 1975, Sargent 1976, Place 1992a). The relative proportions of these lipid classes in copepods vary seasonally and with food abundance (Sargent et al. 1976, Michaud & Taggart 2007). WE are largely stored in an oil sac compartment of copepods (Miller et al. 1998). Stored TAG are separated anatomically from the WE and are utilized much faster (see Sargent et al. 1976). Thus in periods of reduced energy consumption, copepods and other zooplankton are likely to contain higher proportions of WE and fewer TAG. During mid to late summer, C5 copepods will consist almost entirely of WE. Wax esters often contain one or more *cis*- double bonds that lower their melting points (Patel et al. 2001) and enable them to remain liquid in cold, deep waters. This feature may facilitate neutral buoyancy at depth while decreasing energy expenditure and predator interaction (Sargent 1976). Wax esters dominate C5 individuals throughout the diapause until the phytoplankton bloom in early spring and summer, when TAG becomes more prominent to meet the rapid energy needs of maturation and reproductive requirements for egg production (Miller et al. 1998). These TAG most likely reflect lipids accumulated during the spring phytoplankton bloom.

Copepods accumulate lipids both by dietary means and *de novo* synthesis. Whereas TAG stores primarily reflect the dietary uptake of fatty acids from phytoplankton blooms, WE stores are derived from either *de novo* synthesis of fatty alcohols and fatty acids or by dietary uptake of fatty acids derived from phytoplankton (Sargent & Lee 1975). Post-bloom, some TAG fatty acids are reduced to fatty alcohols and are then esterified with a fatty acid to WE (Graeve et al. 2005, Lee et al. 2006).

Several studies have indicated that many marine fish species (Patton & Benson 1975, Patton et al. 1975, Sargent et al. 1979, Mankura et al. 1984, Olsen et al. 2004) and pelagic seabirds (Warham 1977, Jacob 1982, Obst 1986, Roby et al. 1986) metabolize WE, however there has been little work investigating WE metabolism in marine mammals. As North Atlantic right whales in the Bay of Fundy feed almost exclusively on stage V *C. finmarchicus* (Murison & Gaskin 1989, Baumgartner & Mate 2003), they provide an interesting opportunity to examine WE metabolism in a highly derived mammal. Because most mammals are incapable of digesting WE, it is reasonable to predict that mammals foraging on a "waxy" prey may not be able to utilize all of its available energy. It is currently not known whether right whales can digest WE (which can comprise a significant proportion of the potential energy ingested in the form of copepod lipids). Nordøy (1995) suggested that minke whales (*Balaenoptera acutorostrata*) may be capable of digesting WE, by examining lipid class composition of fresh undigested forestomach and colon contents of whales and comparing those measurements with previously determined digestibility estimates of krill using *in vitro* techniques (Nordøy et al. 1993). These data however, were collected from five carcasses, which raise questions about the possibility of the lipid composition being a consequence of post-mortem artifacts.

During the summer in the Bay of Fundy, right whale fecal material has been observed floating after an observed defecation event (Rolland et al. 2007). Thus, it is possible to collect fecal samples and test whether these whales are capable of assimilating all of the potential lipid energy they ingest, using the reasoning that indigestible lipids would pass through the alimentary canal and be expelled as fecal material. The fact that

the feces floats suggests that some lipid may be present, but to date this has not been confirmed chemically nor has any fecal lipid composition work been conducted. If the fecal material does contain elevated amounts of WE and low TAG, then it would suggest that right whales may not be able to metabolize all of the lipids present in their copepod diet. In addition, examining the individual fatty acid and fatty alcohol components that comprise these lipid classes may provide further insight into right whale metabolic processes and the sources of fecal lipids. Current energetic models assume that right whales utilize all of the lipid energy in their ingested prey; therefore, if right whale fecal material is high in WE content, current energetic models may be overestimating right whale metabolic capabilities and underestimating their daily ingestion requirements.

Rolland et al. (2005) have demonstrated that it is possible to obtain physiological information about free-ranging right whales by measuring hormone metabolites in fecal material. This study will determine the lipid metabolic capabilities of right whales by quantifying the total lipid found in copepods and comparing that to the lipid present in sampled fecal material.

The primary objectives of this study were to (i) determine the lipid content and lipid composition of right whale fecal material collected in the Bay of Fundy, (ii) determine the lipid content and lipid composition of right whale's primary prey, stage V copepod, *C. finmarchicus* (iii) evaluate whether right whales were capable of metabolizing all of the lipid in their diet by comparing the lipids a right whale consumes with those that are eliminated in the fecal material, and (iv) estimate daily lipid assimilation of right whales using allometrically-derived right whale ingestion and defecation rates in conjunction with data on copepod and fecal lipid composition. This

study is the first to evaluate WE metabolism in live whales and will provide insights into right whale metabolic capabilities and energetic requirements and may also have implications for the conservation of these highly endangered whales.

MATERIALS AND METHODS

Sample collection

Copepods were collected in the Bay of Fundy over two consecutive summers via weekly plankton tows (2006; n = 56, 2007; n = 51) starting in mid-July each year and continuing through September. Net sampling was conducted in the Grand Manan Basin at two specific locations, which were within an area in which right whales are commonly seen feeding (sightings data collected between 1987 – 2000; NARWC 2008) (see ◊ in Fig. 1). Right whale sightings data collected by the New England Aquarium (NEAq) in 2006 and 2007 (see ○ in Fig. 1) further validate that zooplankton tows were conducted in a habitat where right whales are commonly seen. Tows were carried out in two locations (northern and southern) to account for potential spatial variability in copepod lipid content or densities. Copepod samples were also collected from additional locations (2006; n = 14, 2007; n = 9), located throughout the Bay of Fundy, to test if spatial variation in copepod lipid content or composition existed on a wider scale (see □ and ○ in Fig. 1).

Copepods were collected from a 6 m fiberglass boat using a 61 cm bongo frame attached to two 300 μm mesh nets (Sea-Gear Corporation, Melbourne, Fl). Nets were lowered to the sea floor, then hauled to the surface at approximately 1 m s^{-1} with a davit and hydraulic hauler assembly. Sampling depths were recorded using a depth sounder

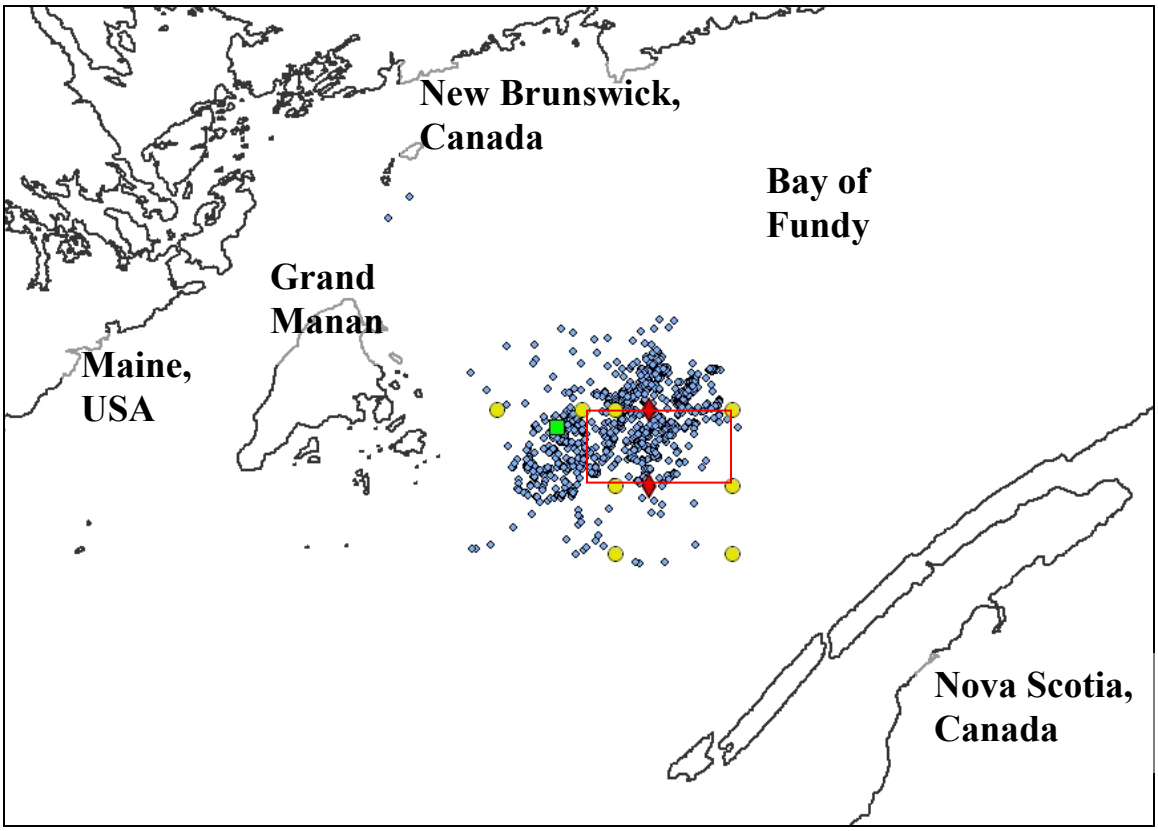

Fig. 1. Map of zooplankton sampling locations in the Bay of Fundy as well as right whale sightings data (●) collected from 2006 and 2007 (NARWC 2008). The red grid outlines an area with the highest probability of seeing a right whale in the Bay of Fundy, based on SPUE (sightings per unit effort) data from 1987 to 2000 (NARWC 2008). The main northern and southern stations are shown (♦) as well as those stations which were sampled less frequently (■, ●).

(Raytheon, Merrimack, NH) and ranged from 190 – 210 m in the northern and southern tow locations. Copepods for species identification and quantification were collected from one of the nets and transferred to 4 % buffered formalin solution. The contents of the second net, used for lipid analysis, were randomly sub-sampled and then placed in a 15 ml cryo-vial (Nalgene, Nunc, Rochester, NY) before being frozen in a 10 L liquid nitrogen dewar (Thermolyne, Barnstead International, Dubuque, Iowa). Macrozooplankton (e.g. euphausiids, pandalids) were removed prior to lipid analysis in order to reduce potential biases introduced by large non-copepod components. These samples were later transferred to a – 80°C freezer until lipid analysis was carried out.

Right whale fecal material was collected in 2006 and 2007 during ship-based whale surveys by the New England Aquarium (NEAq). A 300 μm nylon mesh dipnet (Sea-Gear Corporation, Melbourne, Fl) was used to scoop fecal material from the water as described by Rolland et al. (2005). Fecal samples used for this study were collected by the NEAq between August 9th and September 17th of 2006 (n = 16) and from August 6th through the 21st of 2007 (n = 6). Additional fecal samples (n = 1; 2006, n = 1; 2007) were collected opportunistically by the Grand Manan Whale and Seabird Research Station. All samples were immediately placed in a cooler on board the vessel and subsequently sub-sampled and then stored frozen at approximately – 20ºC until lipid analysis could be conducted.

Zooplankton identification and quantification

Zooplankton samples were well mixed, and using a Folsom plankton splitter and taxonomic keys (Gosner 1971, Smith 1977, Roff 1978, Murphy & Cohen 1979), random

sub-samples (approximately 6 %) were examined and counted under a dissecting microscope to determine species composition, quantity and developmental stages. Zooplankton were identified to genus, species and stage when possible. Zooplankton density (m^{-3}) was calculated for all quantified samples using known depth (m) and net area (0.292 m^2) to determine the volume of water (m^{-3}) sampled. C5 densities were determined by dividing the number of quantified C5 individuals by the volume of water sampled (m^{-3}).

ANOVA ($P < 0.05$ was considered significant) was used to compare differences in zooplankton proportions and densities across sampling month and year. There were not enough copepod tows to statistically compare C5 densities between sampling months within stations, thus C5 densities from the north and south stations were grouped for seasonal comparisons.

Lipid extraction and lipid class determination

Lipids were extracted from fecal and copepod samples using a modified Folch et al. (1957) chloroform: methanol procedure as described in Koopman et al. (1996). Copepods were manually homogenized prior to extraction. Each weighed sample (approximately two grams) was placed in 20 ml of 2:1 chloroform:methanol with 0.01% BHT (butylated hydroxytoluene). The sample was left to soak overnight to ensure complete extraction of lipids. The sample was filtered to remove the solid prey/fecal parts. Methanol was removed by adding 6 ml of 0.7% NaCl, vortexed to ensure thorough mixing, and then centrifuged at 2000 rpm for 10 minutes to separate layers. The methanol water layer was discarded and the chloroform-lipid layer retained. Anhydrous

Na_2SO_4 was added to ensure complete removal of water. The solvent was evaporated under N_2 gas to obtain lipid content (wt%) of the tissue sampled. Lipid was then resuspended in hexane and stored under nitrogen gas at – 20 ˚C until further processing. Lipid classes were identified and quantified by thin layer chromatography with flame ionisation detection (TLC-FID) using an Iatroscan® Mark VI (Mitsubishi Kagaku Iatron, Inc, Tokyo, Japan) (Ackman et al. 1990). Silica gel rods were spotted with samples and developed in a three tank solvent system. Rods were first saturated for approximately 15 min in hexane: diethyl ether (66:4). Rods were then removed, dried and placed in a hexane: ethyl acetate: formic acid (55:15:0.5) solvent for approximately 15 min and then transferred as above to a hexane: benzene solvent (30:30) for approximately 30 min. Rods were then placed in the Iatroscan and scanned at a rate of 30 sec rod^{-1} using 170 ml min^{-1} hydrogen and 2000 ml min^{-1} air flow. Results were integrated with PeakSimple 329 Iatroscan software (SRI Instruments, Torrance, CA). Identification of peaks was confirmed using known standards (NuCheck Prep, Elysian, MN) of WE, TAG, free alcohols, phospholipids, cholesterol and diacylglycerols (described by Koopman et al. 1996). Gas chromatography-mass spectrometry (GCMS) analysis revealed the presence of sterols. Wax esters and sterol esters (SE) coelute on most solvent systems (Parrish & Ackman 1983, Hamilton 1995), therefore WE and SE proportions had to be estimated in the WE/SE peak. To estimate WE and SE proportions in the feces, a WE:SE ratio was calculated from mean FAlc (major component of WE) and mean sterol content (component of SE) obtained from gas chromatography (GC) data (see below). This ratio was applied to the ester peak recorded by the Iatroscan.

ANOVA ($P < 0.05$ was considered significant) was used to compare differences in lipid content and composition in copepod and fecal samples across sampling location, month and year.

Fatty acid and fatty alcohol analyses

Fatty acids (FA) and fatty alcohol (FAlc) components of copepods and feces were identified using GC of butyl esters. Briefly, lipid components were suspended in 50 mg ml^{-1} hexane and BF_3 and then butylated at 100° C. Copepod and fecal FA and FAlc were identified using a Varian capillary GC with FID (Varian Inc, Palo Alto, CA) using a fused silica column (30 x 0.25mm ID) coated with polyimide (0.25µm thickness; J&W Scientific FFAP column; Folsom, CA). Helium was used as the carrier gas (1.0 ml min^{-1}) and the gas line was equipped with an oxygen scrubber. The following temperature program was used: 65°C for 2 min, hold at 165°C for 0.40 min after ramping at 20°C min^{-1}, hold at 215°C for 6.6 min after ramping at 2°C min^{-1}, and hold at 250°C for 10 min after ramping at 5°C/min. Up to 50 different FA and FAlc were identified according to Iverson (1997), Iverson et al. (2002) and Koopman et al. (2006). Fatty acids and fatty alcohols were described using the nomenclature of *A:Bn-X*, where A is the number of carbon atoms, B is the number of double bonds, and X is the carbon position of the double bond closest to the terminal methyl group. These results were integrated with Galaxie (version Varian 1.8.501.1) GC software. Copepod and fecal FA and FAlc components were identified on the same run and then confirmed using prepared standards (NuCheck Prep, Elysian, MN), TLC and GCMS (Trace GC Ultra coupled to a Polar Q

mass spectrometer, Thermo Electron Corporation with X calibur software) (Sue Budge, Dalhousie University).

To determine whether the overall signatures of the copepods and whale feces were different, FA and FAlc data were compared using a multivariate approach. Fifteen dominant FA (16:0, 16:1n-7, 18:0, 18:1n-9, 18:3n-3, 20:0, 20:1n-9, 20:5n-3, 22:1n-11, 22:0, 22:6n-3) and FAlc (16:0, 18:1n-9, 20:1n-9, 22:1n-11) were selected, based on either being the most common components present or being of physiological importance. Fatty acid and fatty alcohol data were arcsine transformed (Steel & Torrie 1980) and screened for outliers. The fifteen FA and FAlc were submitted to a discriminant function analysis (DFA); prior probabilities were unequal (based on sample size). Wilks' Lambda was significant (see results), indicating that feces and copepods were distinguishable. All fecal and copepod cases were then reclassified using discriminant function (DF) scores; leave-one-out classification was also used to determine the robustness of the classification.

Modeling right whale lipid assimilation

To calculate lipid assimilation efficiencies for right whales, total daily ingestion (I_d; number of copepods day^{-1}) was first calculated using a modified ingestion model (Baumgartner & Mate 2003) that incorporated the area of the whale's gape (A_g, m^2), the swimming speed (S, m s^{-1}), duration at depth (T_d, s; actual time feeding) and the copepod concentration available to the whale (C, m^{-3}).

$$I_d = A_g \, S \, T_d \, C \qquad (1)$$

The area of the gape (A_g, 1.21 m^2) was based on a 40000 kg whale (Mayo et al. 2001) and S (1.5 m s^{-1}) was the swimming speed (see Baumgartner & Mate 2003). Duration at depth (T_d, 43 200 s) was based on the assumption that a right whale will spend between 3 – 12 h day^{-1} foraging to meet daily metabolic requirements (as described by Kenney et al. 1986); therefore 12 h day^{-1} was used as a maximum foraging time. The copepod concentration (C) value was based on mean *C. finmarchicus* C5 density (7481 m^{-3}) in the Bay of Fundy as calculated by Baumgartner & Mate (2003). The product of these variables resulted in the daily copepod ingestion value (I_d). Although mean *C. finmarchicus* C5 density estimates from the current study (822.27 ± 94.28 m^{-3}) are similar to other estimated C5 densities (~ 1000 m^{-3}) from the Bay of Fundy (Murison & Gaskin 1989, Woodley & Gaskin 1996, Michaud & Taggart 2007), these estimates were not used in the ingestion model because it has been suggested that a minimum C5 density of 3600 m^{-3} are required for right whales to meet daily metabolic requirements (Baumgartner & Mate 2003). Differences in mean C5 densities between the former (environmental sensing system) and latter studies (net) are likely reflective of the zooplankton sampling method. The latter study's C5 density estimates were dependent on water column-integrated concentrations and may have underestimated C5 densities. Baumgartner & Mate (2003) used an optical plankton counter (OPC) to report a fine-scale estimate of C5 densities in the Bay of Fundy that most likely reflects the spatial distribution of C5 at depth. See Baumgartner (2003) for a discussion of *C. finmarchicus* C5 abundance estimates from nets and optical plankton counters.

To convert I_d to total grams ingested day^{-1} (I_g, g), individual *C. finmarchicus* C5 (n=20) were weighed and a mean wet weight of 1.47 mg indiv^{-1} was calculated and multiplied by I_d.

$$I_g = I_d\ (1.47\ \text{mg C5 indiv}^{-1}) \quad (2)$$

Total lipid ingested day^{-1} (I_L, g) was determined by multiplying I_g by the mean *C. finmarchicus* C5 percent lipid content value of 7.17 wt% (mean lipid content value measured from the current study).

$$I_L = I_g\ (7.17\ \text{wt\%}). \quad (3)$$

Copepod lipid class data (mean percent of total lipid) were then multiplied by I_L to estimate the amount (g) of each lipid class ingested day^{-1}.

There are very few published data on defecation rates of wild mammals, let alone for marine mammals, therefore it is difficult to estimate the daily fecal production in right whales. Allometric modeling is a useful tool for predicting various ecological and physiological characteristics from body weight (W, g) (e.g. White & Seymour 2005, Duncan et al. 2007). Based on allometric models provided by Blueweiss et al. (1978) and Lavigne (1982), combined with fecal lipid content data, it was possible to model approximately how much lipid an average right whale (W, 40000 kg) eliminates on a daily basis. Blueweiss et al. (1978) provided an equation estimating mammal defecation rates (F, g g^{-1} day^{-1}) in relation to body size, where:

$$F = 0.85\ W^{-0.37} \quad (4)$$

Lavigne (1982) modified this equation to report total fecal production (FE) on a whole animal basis (g day^{-1}) where:

$$FE = 0.85\ W^{0.63}. \quad (5)$$

It was assumed that an average right whale weighs 40 000 kg (Kenney et al. 1986), therefore by using equations (4) and (5) it was possible to estimate daily fecal production (g feces day^{-1}). To determine the amount of lipid a right whale eliminates as fecal material (g lipid day^{-1}), mean fecal percent lipid data from 2006 and 2007 were multiplied by the estimated right whale fecal production rate. Fecal lipid class data were multiplied by the mean percent lipid content to determine the amount of each component eliminated.

Right whale ingestion and defecation rates were then compared to estimate the amount of lipid assimilated. Ingestion and defecation rates were converted to lipid rates (g day^{-1}) and then compared to estimate the amount of lipid that was assimilated per day. Lipid content and class composition were then multiplied by the lipid ingestion and defecation rates to determine the amount of each component that was assimilated per day.

All statistical analyses were carried out using SPSS (SPSS Inc., Chicago, IL). All means are presented ± standard error (SE).

RESULTS

Zooplankton identification and species composition

The dominant zooplankton species present in each tow (n = 42 total samples) collected for quantification in 2006 and 2007 from the northern (n = 17), southern (n = 20) and basin-wide (n = 5) tow sites was *C. finmarchicus* (Table 1). In both years, stage V *C. finmarchicus* (C5) dominated all zooplankton samples (2006: 83.5 ± 1.7 %; 2007: 71.1 ± 2.3 %) (Tables 1, 2) with a significantly smaller percentage of C5 in 2007 ($P = 0.004$). In 2006, there were no significant differences (all $P > 0.05$) observed in C5

Table 1. Mean zooplankton composition (% frequency of occurrence) of samples collected at the northern and southern tow stations (◊) from July through September of 2006. One tow (other, □) in September was conducted outside of the grid to determine any spatial variability in zooplankton composition. Numbers in parentheses represent the number of tows conducted at each station. All means are ± standard error.

	2006						
	Location	North (◊)			South (◊)		Other (□)
Taxa	July (1)	Aug (2)	Sept (5)	July (1)	Aug (6)	Sept (5)	Sept (1)
C. finmarchicus (V)	92.98	85.38 ± 1.19	83.98 ± 1.58	90.21	80.90 ± 3.21	80.37 ± 5.02	91.12
C. finmarchicus (adult)	0.14	6.41 ± 0.56	6.57 ± 1.09	4.46	5.26 ± 1.04	4.25 ± 1.13	1.44
C. finmarchicus (IV)	0.62	1.66 ± 0.07	1.77 ± 0.81	1.90	4.35 ± 2.13	3.27 ± 1.77	0.54
C. finmarchicus (III)	--	0.15	0.11 ± 0.07	--	2.01 ± 1.75	0.66	0.22
C. hyperboreous	2.40	4.15 ± 0.42	1.07 ± 0.10	1.61	1.82 ± 0.34	1.08 ± 0.24	0.74
C. glacialis	--	0.23	0.43 ± 0.23	--	0.17 ± 0.08	0.47 ± 0.17	0.24
Metridia	1.43	0.71 ± 0.02	0.68 ± 0.16	1.68	1.64 ± 0.38	1.35 ± 0.39	0.22
Centropages	--	--	2.76 ± 0.49	--	0.06	3.77 ± 1.36	3.22
Arcatia tonsi	0.19	0.51 ± 0.40	2.02 ± 0.75	--	4.08 ± 1.88	4.39 ± 3.28	2.26
Pseudocalanus	--	--	--	--	--	--	--
Euchaeta	2.24	0.72 ± 0.14	0.41 ± 0.14	0.15	0.85 ± 0.19	0.77 ± 0.37	--

Table 2. Mean zooplankton composition (% frequency of occurrence) of samples collected at the northern and southern tow stations (◊) from July through September of 2007. Four tows (other, ○) in September were conducted outside of the grid to determine any spatial variability in zooplankton composition. Numbers in parentheses represent the number of tows conducted at each station. All means are ± standard error.

	2007						
	Location	North (◊)			South (◊)		Other (○)
Taxa	July (4)	Aug (4)	Sept (2)	July (3)	Aug (6)	Sept (2)	Sept (4)
C. finmarchicus (V)	56.32 ± 5.78	77.71 ± 3.51	80.67 ± 2.95	65.62 ± 2.56	72.82 ± 2.57	87.76 ± 0.08	67.77 ± 6.35
C. finmarchicus (adult)	10.89 ± 1.12	6.34 ± 0.85	7.07 ± 1.41	7.35 ± 1.35	8.61 ± 0.95	5.32 ± 1.14	12.93 ± 2.36
C. finmarchicus (IV)	10.15 ± 6.51	0.18 ± 0.03	0.25	11.61 ± 6.45	1.20 ± 0.52	0.07 ± 0.02	1.41 ± 1.01
C. finmarchicus (III)	1.56 ± 1.33	0.07	0.05	0.94 ± 0.03	0.37 ± 0.13	0.08	0.56
C. hyperboreous	2.75 ± 0.72	2.57 ± 0.63	3.35 ± 1.07	1.56 ± 1.08	2.53 ± 0.38	1.12 ± 0.44	1.28 ± 0.49
C. glacialis	8.27 ± 2.63	8.80 ± 1.66	5.09 ± 2.72	4.68 ± 1.25	6.97 ± 0.54	3.87 ± 0.98	5.19 ± 1.89
Metridia	2.45 ± 0.52	1.56 ± 0.53	1.34 ± 0.58	1.68 ± 0.56	1.28 ± 0.24	0.53 ± 0.23	2.22 ± 1.20
Centropages	--	--	--	--	--	--	1.84 ± 0.54
Arcatia tonsi	7.58 ± 0.43	1.92 ± 0.66	1.45 ± 0.22	6.56 ± 1.46	5.95 ± 1.79	1.01 ± 0.56	7.85 ± 2.11
Pseudocalanus	0.30	--	--	--	--	--	--
Euchaeta	0.47 ± 0.24	0.87 ± 0.37	0.87 ± 0.09	0.32 ± 0.08	0.41 ± 0.23	0.28 ± 0.19	0.49 ± 0.08

proportion between sampling stations or months. In 2007, there was an increased trend in C5 proportion from July to September, with significantly lower percentage of C5 observed in July ($P = 0.000$), however there were no significant differences between August and September ($P > 0.05$, Tables 1, 2). Other species present included *C. hyperboreous*, *C. glacialis*, *Arcatia tonsi* and *Metridia*. Of these species, *C. glacialis* and *Arcatia tonsi* showed the most annual variability with increased proportions from 2006 to 2007 (Tables 1, 2). Larger zooplankton consisted of euphausiids, pandalids and cnidarians, however these species were infrequent and represented less than 0.1 % of the individuals in a tow.

Zooplankton Density

C5 densities were greatest in samples collected from the northern and southern stations (as compared to stations outside of the grid; see below), with mean overall C5 densities of 788.74 ± 92.83 mP^{-3} in 2006 and 894.24 ± 102.26 mP^{-3} in 2007. There were no significant annual differences in C5 densities ($P > 0.05$) at either the northern and southern stations (Table 3). There was a seasonal trend toward increased C5 densities at both the northern and southern stations, with the highest concentrations observed in September (2006; 935.15 ± 132.14 m^{-3}, 2007; 1258.91 ± 245.81 m^{-3}). Yet, there were no significant seasonal differences ($P > 0.05$) in C5 densities observed between months in either 2006 or 2007 (Table 3). Zooplankton samples obtained from outside the grid ($n = 5$) were primarily collected in 2007 and showed no significant density differences ($P > 0.05$) between sampling sites. Most C5 densities that were collected outside of the grid (mean 193.43 ± 84.66 m^{-3}) were significantly lower ($P = 0.002$) than samples collected

Table 3. Mean C5 densities (m^{-3}) from the northern and southern tow stations (◊) from July through September of 2006 and 2007. One tow (other, □) in September 2006 and four tows (other, ○) in September 2007 were conducted outside of the grid to measure any spatial variability in zooplankton composition. Numbers in parentheses represent the number of tows conducted at each station. All means are ± standard error.

	2006			2007		
Location	July	Aug	Sept	July	Aug	Sept
North (◊)	419.23 (1)	788.14 ± 70.78 (2)	1062.01 ± 189.86 (5)	361.80 ± 201.78 (3)	1073.12 ± 177.53 (4)	977.11 ± 90.29 (2)
South (◊)	271.99 (1)	644.57 ± 171.79 (4)	808.30 ± 185.69 (5)	854.13 ± 142.71 (3)	802.94 ± 165.76 (5)	1540.70 ± 442.22 (2)
North & South (◊)	345.61 ± 73.62 (2)	692.43 ± 114.26 (6)	935.15 ± 132.14 (10)	607.96 ± 156.00 (6)	923.02 ± 123.04 (9)	1258.91 ± 245.81 (4)
Other (□, ○)	--	--	2612.53 (1)	--	--	193.43 ± 84.66 (4)

from within the grid (northern and southern sites) (mean 842.92 ± 68.86 m^{-3}; Table 3). However, on September 27th 2006, a sample collected outside of the grid and adjacent to a group of feeding right whales, had a C5 density (2612.5 mP^{-3}) that was more than twice the mean values from all other samples collected during this study (Table 3).

Lipid content and composition of copepods

Copepod lipid content averaged 7.17 % ± 0.14 wt% (wet weight) in all sampling sites during 2006 and 2007. Although there was a wide range in lipid content (4.55 to 9.91 wt%), there were no significant differences ($P > 0.05$) across months, years or sampling sites (Fig. 2).

The dominant lipid class present among all copepod samples was wax ester (WE), followed by phospholipid (PL), triacylglycerol (TAG), free fatty acid (FFA), and cholesterol (Chol) (Figs. 3, 4). There was little seasonal variation in lipid class composition in samples collected in either 2006 or 2007. On average, copepods contained 93.75 ± 0.30 % WE and showed no significant differences ($P > 0.05$) in WE content across months, years or sampling sites (Figs. 3, 4). Phospholipid ranged from approximately 2 – 5 % (mean 3.56 %) from samples collected in 2006 and 2007, however there were no significant spatial or temporal differences observed ($P > 0.05$; Figs. 3, 4). Mean TAG content was 1.52 ± 0.07 %, and although there was a slight decrease in TAG from July to September of 2007 from approximately 2.0 to 1.5 %, there were no significant spatial or temporal differences ($P > 0.05$) in TAG components (Figs. 3, 4). FFA and Chol averaged < 1.0 % for all samples collected and showed no significant differences ($p > 0.05$) across sampling month, year or location (Figs. 3, 4). GCMS

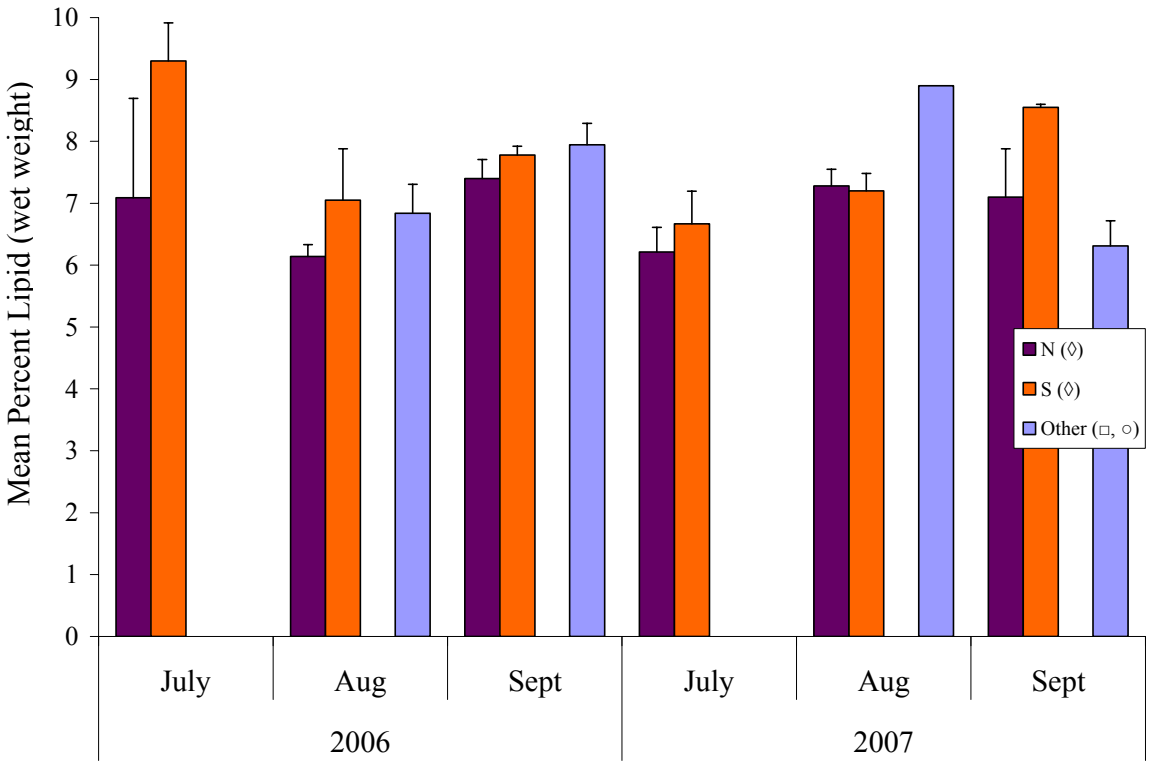

Fig. 2. Mean C5 lipid content values (wet weight) from zooplankton samples collected in 2006 and 2007 from the northern (N), southern (S), and all stations (other) outside of the grid.

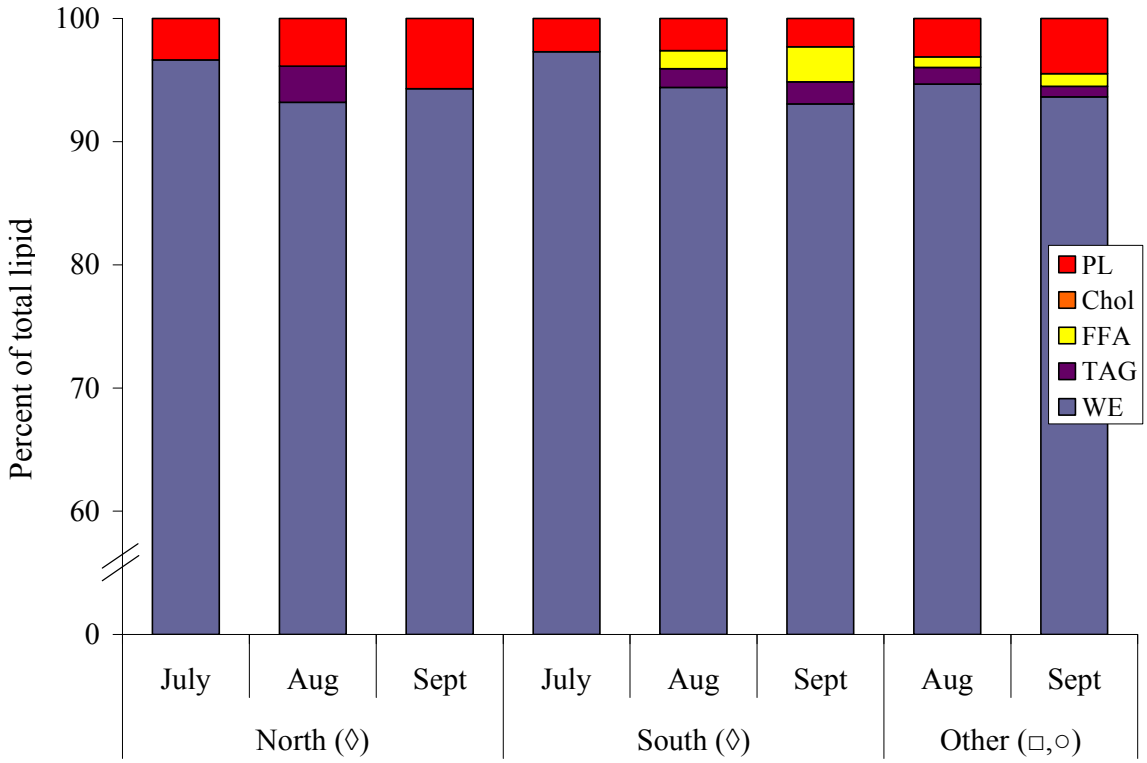

Fig. 3. Mean copepod lipid class composition (% of total lipids) from 2006 in samples collected from the northern, southern and all stations (○ & □) outside of the grid. (WE, wax ester; TAG, triacylglycerol; FFA, free fatty acid; Chol, cholesterol; PL, phospholipid).

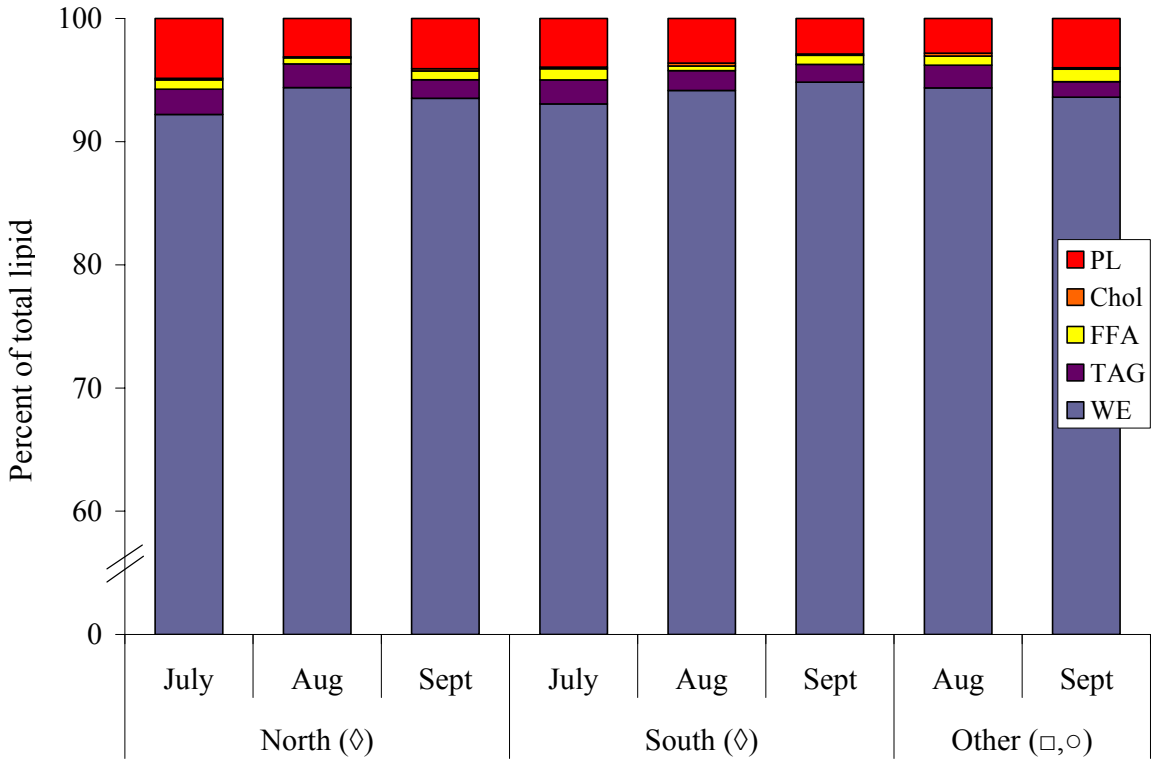

Fig. 4. Mean copepod lipid class composition (% of total lipids) from 2007 in samples collected from the northern, southern and all stations (○ & □) outside of the grid. (WE, wax ester; TAG, triacylglycerol; FFA, free fatty acid; Chol, cholesterol; PL, phospholipid).

confirmed the absence of sterol esters in copepods.

Copepod samples collected throughout the Bay of Fundy in 2006 and 2007 consisted primarily of long chain fatty acids (FA) and fatty alcohols (FAlc). Specifically, 14:0 and 16:0 were the dominate FA components in all copepod samples, with monounsaturated and polyunsaturated components 16:1n-7, 18:1n-9, 20:1n-9, 22:1n-11 and 18:4n-3, 20:5n-3, 22:6n-3 comprising the majority of the remaining FA components (Table 4). The long chained monounsaturates 20:1n-9 and 22:1n-11 dominated the FAlc composition, at 10 – 12 wt% of the total lipid, followed by the 16:0 and 22:1n-9 FAlc components (Table 4).

Lipid content and composition of right whale feces

In 2006 and 2007, right whale fecal percent lipid averaged 2.48 ± 0.43 wt% and 4.29 ± 0.39 wt% and ranged between 0.9 and 8.6 wt%. There were no significant differences in fecal percent lipid between sampling months in 2006 and 2007, however fecal percent lipid was significantly higher in 2007 ($P = 0.020$).

Due to WE and sterol ester (SE) coelution in most solvent systems, WE and SE proportions in the feces were estimated using a WE:SE ratio as calculated from mean FAlc and mean sterol content obtained from GC data. This ratio was applied to the ester peak recorded by the Iatroscan. The dominant lipid classes present in all fecal samples collected in 2006 and 2007 were TAG and SE, followed by WE, PL, Chol, FFA and unknown lipid components. Across all samples, mean estimated TAG and SE lipid class composition were 29.17 ± 2.48 and 31.34 ± 2.75 wt%, respectively, and showed no significant differences ($P > 0.05$) in fecal lipid class content between sampling months or

Table 4. Mean fatty acid and fatty alcohol composition (wt%) of copepods collected in 2006 (n = 38) and 2007 (n = 26). Although 66 FA and 11 FAlc were identified, only components representing > 0.2 wt % are shown. All means are ± standard error.

Saturated, monounsaturated & polyunsaturated components	2006		2007	
	FA	FAlc	FA	FAlc
	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
14:0	12.7 ± 0.20	0.61 ± 0.05	17.06 ± 0.23	0.73 ± 0.08
15:0	0.60 ± 0.01	ND	0.80 ± 0.01	ND
16:0	7.93 ± 0.15	2.67 ± 0.08	10.5 ± 0.14	2.93 ± 0.11
16:1n-7	4.17 ± 0.09	0.62 ± 0.09	6.01 ± 0.17	0.29 ± 0.05
16:1n-5	ND	ND	0.54 ± 0.01	ND
16:3n-6	0.57 ± 0.01	ND	ND	ND
16:4n-1	0.55 ± 0.03	ND	0.85 ± 0.06	ND
18:0	0.58 ± 0.03	ND	0.48 ± 0.02	ND
18:1n-9	3.36 ± 0.07	0.74 ± 0.05	3.97 ± 0.10	ND
18:1n-7	ND	0.53 ± 0.03	0.53 ± 0.02	ND
18:2n-6	0.90 ± 0.05	ND	1.12 ± 0.01	ND
18:3n-3	1.00 ± 0.02	ND	1.34 ± 0.01	ND
18:4n-3	4.48 ± 0.07	ND	6.46 ± 0.17	ND
20:1n-11	0.89 ± 0.04	ND	1.33 ± 0.10	ND
20:1n-9	3.73 ± 0.11	9.70 ± 0.17	6.02 ± 0.24	4.69 ± 0.81
20:1n-7	ND	1.06 ± 0.04	ND	0.85 ± 0.17
20:4n-3	0.58 ± 0.01	ND	0.77 ± 0.02	ND
20:5n-3	5.59 ± 0.13	ND	8.00 ± 0.24	ND
22:1n-11	7.05 ± 0.08	12.28 ± 0.40	8.44 ± 0.15	2.29 ± 0.40
22:1n-9	0.51 ± 0.02	3.10 ± 0.18	0.6 ± 0.02	0.20 ± 0.04
22:6n-3	5.26 ± 0.09	ND	5.33 ± 0.12	ND
24:1n-9	0.45 ± 0.01	0.74 ± 0.06	0.49 ± 0.01	0.10 ± 0.01

ND – Component not detected.

years (Fig. 5). Similary, fecal WE composition at 16.92 ± 3.40 wt% (wet weight) showed no annual or monthly differences ($P > 0.05$).

Right whale fecal composition was dominated by saturated FA components, consisting primarily of 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 22:0 and 24:0 (Table 5). Monounsaturated FA 18:1, 20:1, 22:1, 22:1 and 24:1 comprised the majority of the FA remaining in the feces. Fatty alcohol, sterol, anteiso (*ai*-) and iso (*i*-) components each represented < 1.0 wt% of the fecal composition. Unlike the copepods, there were no polyunsaturated fatty acids (PUFA) observed in the fecal material.

The overall FA and FAlc compositions of the copepods and feces were very different. The DFA showed Wilks' Lambda to be significant ($P < 0.000$), indicating that the two types of samples could be distinguished using lipid components. Because only two groups were used, the DFA yielded a single DF. Classification based on DF scores resulted in all cases being correctly classified. Validation of this approach using leave-one-out classification also resulted in 100 % correct classification of all cases into their original grouping.

Modeling lipid assimilation

To address sampling biases associated with the net sampling technique used in the current study, a mean C5 density of 7481 m^{-3} (Baumgartner & Mate 2003) was used to estimate daily ingestion rates. C5 ingested day^{-1} (I_d) was estimated as 586570248 indiv C5 day^{-1}. Grams of C5 ingested day^{-1} (I_g) were estimated as 862258 g C5 day^{-1}. Assuming mean C5 percent lipid content of 7.17 %, grams of lipid ingested day^{-1} (I_L) were estimated as 61824 g lipid day^{-1} (Table 6). Wax ester was the dominate lipid

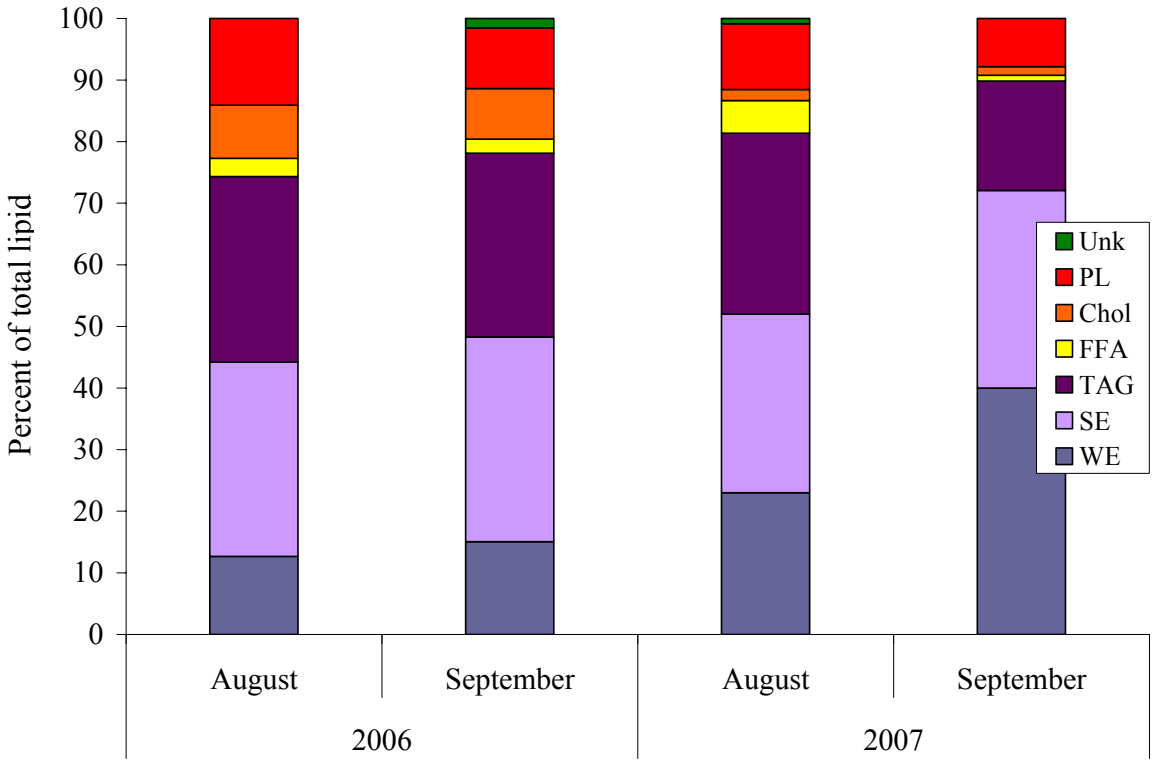

Fig. 5. Mean right whale fecal lipid class composition (% of total lipids) by month from fecal samples collected in 2006 and 2007. (WE, wax ester; SE, sterol ester; TAG, triacylglycerol; FFA, free fatty acid; Chol, cholesterol; PL, phospholipid; Unk, unknown components).

Table 5. Fatty acid (FA), fatty alcohol (FAlc), sterol ester (SE) and ante/iso composition (wt%) of right whale feces. Sterol and anteiso and iso values are sums of all components. All means are ± standard error.

Saturated & monounsaturated components	2006		2007	
	FA Mean ± SEM	FAlc Mean ± SEM	FA Mean ± SEM	FAlc Mean ± SEM
12:0	0.25 ± 0.03	--	0.18 ± 0.03	--
14:0	5.35 ± 0.42	0.16 ± 0.19	5.22 ± 0.52	0.05 ± 0.02
15:0	3.01 ± 0.21	--	2.09 ± 0.25	--
16:0	22.05 ± 1.07	0.01 ± 0.001	22.15 ± 0.93	0.01 ± 0.001
17:0	2.17 ± 0.19	--	2.36 ± 0.27	--
18:0	15.89 ± 1.25	--	16.03 ± 1.64	--
18:1	2.49 ± 0.30	0.06 ± 0.02	3.11 ± 0.79	0.09 ± 0.02
19:0	0.52 ± 0.05	--	0.46 ± 0.10	--
20:0	7.98 ± 0.46	--	8.58 ± 0.74	--
20:1	5.63 ± 0.49	0.25 ± 0.09	6.61 ± 1.23	0.74 ± 0.42
21:0	0.21 ± 0.03		0.30 ± 0.03	
22:0	11.77 ± 1.59	--	9.31 ± 1.44	--
22:1	10.54 ± 1.16	0.26 ± 0.18	10.31 ± 1.05	1.31 ± 0.66
24:0	1.08 ± 0.14	0.36 ± 0.23	1.38 ± 0.42	0.57 0.43
24:1n-9	2.35 ± 0.24	--	1.99 ± 0.32	--
Unknown	1.57 ± 0.17	--	2.33 ± 0.74	--
Sterols	2.19 ± 0.28	--	1.88 ± 0.39	--
Anteiso and iso	3.50 ± 0.21	--	0.51 ± 0.25	--

Table 6. Mean lipid class ingested and eliminated (g day^{-1}) based on mean percent copepod and fecal lipid class values (wt%). Lipid classes assimilated based on percent lipid classes assimilated and lipid assimilation values (g).

		Lipid class						
% lipid	Lipid ingested (g) day^{-1}	WE	SE	TAG	FFA	Chol	PL	Unk
7.2	61824	58013	ND	1036	494	87	2194	ND
	Feces eliminated (g) day^{-1}							
3.0	1570	248	515	461	61	86	182	17
	Lipid assimilated (g) day^{-1}							
	60786	57765	--	575	433	1	2012	--

component ingested by right whales, at 58013 g (mean) of the total daily lipid ingested (Table 6). Other lipid components represented a smaller percentage of the total lipid ingested.

Using the allometric relationship to determine fecal production rates (FE = 0.85 $W^{0.63}$), a 40000 kg right whale was estimated to eliminate 52326 g feces day^{-1}. Based on these data and mean fecal lipid content (3.0 %), it was estimated that a right whale would eliminate 1570 g lipid day^{-1} (Table 6).

Triacylglycerols and sterol esters were the dominant lipid classes eliminated day^{-1} (Fig. 5, Table 6), comprising 29 % and 33 %, respectively, of the total lipid in feces at 461 g day^{-1} and 515 g day^{-1}. Phospholipid and wax ester components consisted of 12 % and 16 %, respectively, of the eliminated lipid (PL: 182 g day^{-1}; WE: 248 g day^{-1}). The other lipid components combined represented approximately 10 % of the total lipid eliminated (Table 6).

Total grams of copepods assimilated was estimated as 809932 g day^{-1}. Grams of lipid assimilated per day were estimated as 60786 g day^{-1} (Table 6). Daily WE assimilation was estimated at 57765 g day^{-1}, followed by TAG and PL components at 575 g day^{-1} and 2012 g day^{-1} (Table 6). These data represent a total (mass-based) assimilation efficiency of 94%, a lipid assimilation efficiency of 98% and a WE assimilation efficiency greater than 99% (Fig. 6).

DISCUSSION

Wax ester metabolism

It is evident from this study that right whales are assimilating the majority of the

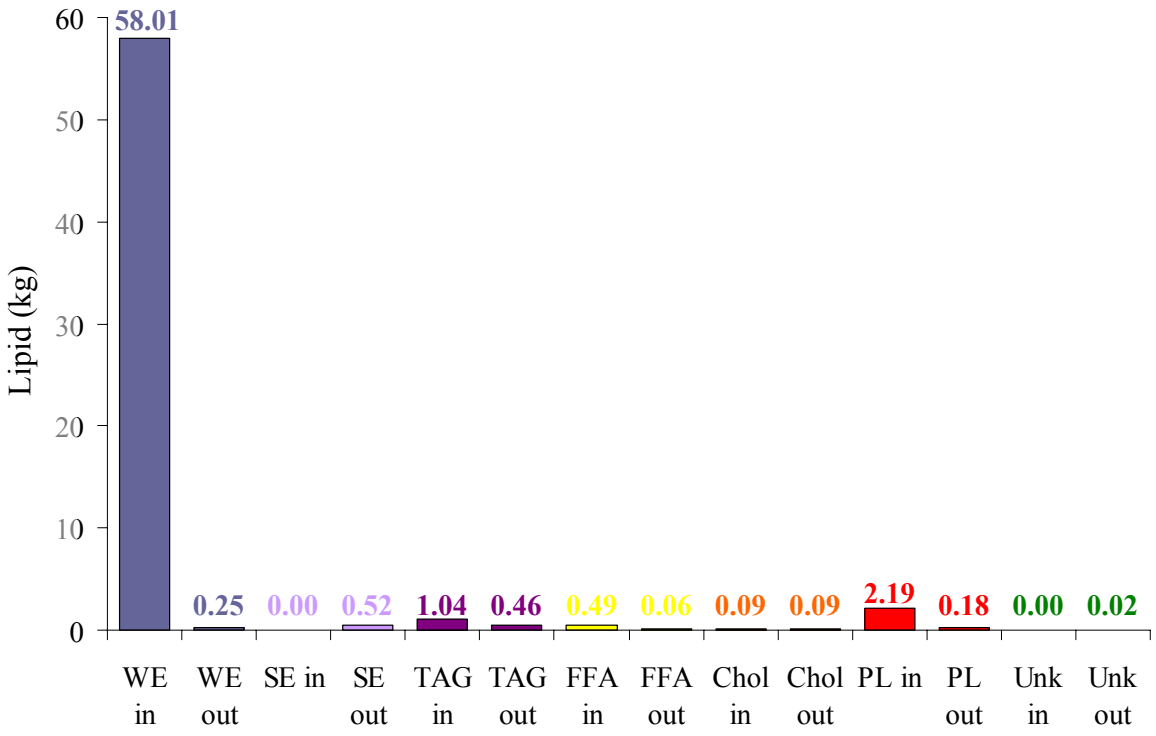

Fig. 6. Lipid class ingestion and elimination rates (kg day^{-1}) as estimated for right whales. (WE, wax ester; SE, sterol ester; TAG, triacylglycerol; FFA, free fatty acid; Chol, cholesterol; PL, phospholipid; Unk, unknown components).

lipid from their copepod diet. Wax esters are the dominant lipid present in the copepod prey but are present in extremely small quantities in right whale fecal material. These data, along with low overall lipid content in right whale feces, indicate that right whales are able to assimilate the WE components of the copepods they ingest. Fatty acid and fatty alcohol analyses further validate WE assimilation in right whales, and indicate that the majority of the lipid components in the feces are not of dietary origin.

In marine systems, lipids originate at different trophic levels, therefore to understand the origin of right whale fecal lipid components, one must first understand the trophic pathway of marine lipids. Phytoplankton synthesize all of their FA *de novo*, which primarily consist of PUFA (Sargent 1976). Zooplankton are unable to synthesize PUFA *de novo* and therefore must obtain polyunsaturated components from their diets. Zooplankton and all other marine animals rich in WE can synthesize some FA components *de novo* (see Sargent 1976). FA synthesized *de novo* or of dietary origin are reduced to their FAlc constituents and then esterified to a FA to form a WE (Sargent 1976), allowing rapid WE synthesis during food-rich periods (Lee et al. 1971). In zooplankton, WE components primarily consist of saturated and monounsaturated FA and FAlc, and although PUFA compose a portion of zooplankton lipid composition, there are no polyunsaturated fatty alcohols (PUFAlc). Fatty acid and fatty alcohol components found in copepods were not seen in the same quantities in the fecal material. FAlc components (a primary component of WE) in the feces consisted of less than 2 % of the total lipid, reflecting the overall low quantities of WE in the feces. In addition, many of the saturated FA components (15:0, 18:0, 20:0 and 22:0) found in the feces (Table 5)

were not present in the copepods (Table 4), suggesting they came from another source (see below).

Nordøy (1995) presented evidence that minke whales (*B. acutorostrata*) may be capable of digesting 92 % of the WE in the krill (*Thysanoessa inermis*) they consume by analyzing the lipid components of fresh undigested forestomach and colon contents and comparing these to previously determined digestibility estimates of krill using *in vitro* techniques (Nordøy et al. 1993). These studies reported results from just five minke whale carcasses and were unable to fully address whether WE breakdown occurred pre- or postmortem. The present study overcame these limitations by comparing lipid components in freshly sampled copepods (n = 60) and fecal material collected from live whales (n = 24) over a two year period.

Although WE in marine environments have been studied for several decades (see Lee et al. 2006), the role of WE as an energy store is unclear. TAG can be rapidly metabolized by all animals, and thus is the primary form of ingested and stored lipids in all mammals. Evidence from *in vitro* and *in vivo* studies indicates that most animals, mammals in particular, are extremely inefficient at metabolizing waxes, as they hydrolyze WE at only about one-tenth the rate that TAG can be broken down (Savory 1971, Patton & Benson 1975, Sargent 1976, Place 1992a). Thus, the majority of WE passes through the gastrointestinal system undigested and is eliminated as part of the fecal material. Mammals are thought to have a WE assimilation efficiency of < 50 % (Hansen & Mead 1965, Place 1992a). Several studies have shown that many fish species such as herring, trout and salmon utilize WE (Patton & Benson 1975, Patton et al. 1975, Sargent et al. 1979, Mankura et al. 1984, Olsen et al. 2004). Olsen et al. (2004) showed

efficient WE assimilation by Atlantic salmon, *Salmo salar*, after comparing fecal lipid contents to ingested lipid from *C. finmarchicus*. Similarly, some seabirds (e.g. most members of the pelagic seabird order Procellariiformes) take advantage of the WE components of their diets (Warham 1977, Jacob 1982, Obst 1986, Roby et al. 1986). Roby et al. (1986) used isotopic markers to investigate WE assimilation in one alcid (*Aethia pusilla*) and three petrels (*Pelecanoides urinatric, P. georgicus* and *Pachyptila desolata*) by analyzing ingested dietary markers in stomach, intestinal and fecal contents and found that chicks of the four species efficiently hydrolyze wax esters.

Several mechanisms to digest WE have been postulated, including bile-activated lipase, and increased gut retention time via a pyloric caeca (Patton & Benson 1975, Place 1992b). Friedman & Kern (1956) first suggested that the lesser honeyguide (*Indicator minor*) contains a symbiotic gut microorganism to help breakdown WE. Downs et al. (2002) later analyzed the microbial fauna in the honeyguide's gastrointestinal cavity and using enzymatic assays, found no microbial activity in the same species and instead suggested a decreased gut passage rate increases the time that digesta are in contact with enzymes. Several delphinid species of the genus *Stenella* and other whale species (minke, sperm whales (*Physeter macrocephalus*) and bowheads (*Balaena mysticetus*)) eat WE rich prey (Gaskin 1982) however, little work has been done to investigate the digestive capabilities of these predators. Several studies have examined microbial activity in forestomachs of minke (Nordøy et al. 1993, Olsen et al. 1994, Nordøy 1995, Olsen et al. 2000), bowhead, gray whales (*Eschrichtius robustus*) (Herwig et al. 1984) and fin whales (*Balaenoptera physalus*) (Herwig & Staley 1986). These studies report high microbial activity in the forestomach, suggesting the multi-chambered stomach may

act as a fermentation center, extending digesta retention times. Olsen et al. (2000) reported that the forestomach of minke whales contain chinolytic bacteria which are capable of breaking down the chitinous shell (indigestible in most mammals) of their crustacean prey (krill). As mentioned above, Nordøy (1995) reported a WE assimilation efficiency of 92 % in minke whales and suggested the possibility that a gut microorganism may enable these processes. Although many studies (see above) have reported evidence of WE assimilation by several different species, there remains a lack of direct evidence of the mechanisms that may allow efficient WE assimilation. It is likely that different taxa utilize different mechanisms to accomplish this goal. This would be an area of research worth further investigation.

Several species use alternative metabolic mechanisms to breakdown the primary components in their diet that would otherwise be indigestible. Animals such as cattle, horses, sheep, goats and termites are metabolically incapable of breaking down the cellulose in their diet, but take advantage of enzymes produced by a symbiotic bacteria to break down the carbohydrate-rich resource (Randall et al. 2002). In cattle and termites, cellulase, a digestive enzyme capable of breaking down cellulose and hemicellulose, is produced either intracellularly (cattle) or extracellularly (termites) by a gut symbiont that allow cattle and termites to absorb the resultant digested fragments (Randall et al. 2002). It is possible that right whales and other large marine grazers use an analogous mechanism to that of ruminants to take advantage of ingested WE.

Wax esters primarily consists of long chained FA and FAlc, of which, the FA components (FAlc oxidized to FA) could be rapidly transported from the intestine to the blood stream. Right whale fecal lipid composition did not reflect the lipid composition of

the copepods or of typical mammalian endogenous lipids. In fact, there was no overlap whatsoever between FA and FAlc signatures of copepods and feces, as demonstrated by DFA. The fecal composition consisted of > 60 % saturated FA components, of which 17:0, 18:0, 20:0, 22:0 and 24:0 were not found in any significant quantities in the copepods (Table 5). Fatty alcohol components consisted of < 2 % (Table 5) of the fecal composition supporting the absence of WE in the fecal material. Sterol ester components were present in fecal material, although in very small amounts of < 0.5 wt% (Table 5). Currently, the origin of the saturated lipid components found in the feces of right whales is unknown. It is unlikely that right whales would selectively ignore saturated components of dietary origin, as they are easily broken down for metabolism or lipid storage and represent considerable energy stores. The large proportion of saturated FA in the feces suggests that they may originate in the gut, however their exact origin is still unclear. Mammals typically synthesize 16:0, 18:0, 16:1 and 18:1, but not 20:0, 22:0 and 24:0 (Iverson 1993). Bacterial organisms, however, can produce saturated FA components, including 20:0, 22:0 and 24:0 (O'Leary 1962). The presence of a gut microorganism capable of digesting WE would represent an unusual mammalian physiological adaptation that allows right whales to take advantage of an abundant food resource.

Identity and distribution of right whale prey in the Bay of Fundy

In the Bay of Fundy, *C. finmarchicus* dominates the zooplankton community throughout the summer and early fall, with Stage V (C5) individuals comprising the majority of *Calanus* (Table 1). Although the percentage of C5 was significantly lower in

2007 than in 2006 (P = 0.004), the apparent decrease in C5 proportion between years is likely reflective of an increase in abundance of other zooplankton species present in the sample (Table 2). This hypothesis is supported by the uniform C5 density values obtained for 2006 and 2007 (Table 3). An increased trend toward higher proportions of C5 from July to September, along with corresponding lower proportions of stage III and IV, is reflective of *Calanus* developmental life phases, as the majority of C5 individuals enter a diapause phase prior to winter months (Hirche 1996). Data from this study, along with almost two decades of zooplankton survey data from the Bay of Fundy (Murison & Gaskin 1989, Baumgartner & Mate 2003, Michaud & Taggart 2007), support the gradual increase and dominance of C5 individuals during late summer and early fall.

C5 densities were greatest in the regions associated with high right whale sightings in 2006 and 2007 (NARWC 2008, Table 3) which supports the contention that the Grand Manan Basin represents an important feeding habitat for right whales (Murison & Gaskin 1989, Baumgartner & Mate 2003, Michaud & Taggart 2007). The lack of variation observed in C5 densities between the northern and southern tow locations (northern, 861.04 ± 100.22 m^{-3}; southern 827.51 ± 96.97 m^{-3}) suggest that these areas are relatively homogeneous, and thus likely represent similar, predictable feeding habitats for right whales. Data from this study and several others conducted in the Bay of Fundy as early as 1989 (Murison & Gaskin 1989, Michaud & Taggart 2007) have reported similar C5 densities of approximately 1000 C5 m^{-3}, with the highest concentrations found in the vicinity of feeding whales. These data suggest that in late summer and early fall, parts of the Bay of Fundy, primarily the deep water regions greater than > 100 m, are highly

predictable foraging areas for right whales (Murison & Gaskin 1989, Baumgartner & Mate 2003).

Tows conducted at sites outside the main region (Fig. 1) contained significantly lower C5 densities in late summer and early fall, which suggests that certain areas within the Bay of Fundy may be less important feeding areas. However, an opportunistic tow conducted on September 27th 2006 outside of the grid, but among a group of diving whales (see Fig. 1, □), contained the highest C5 density (2867.83 m^{-3}) collected during this study. Although this tow was conducted in relatively deep water (191 m) and in close proximity to the selected grid, it supports the idea that water depth is an important parameter for copepod accumulation. These data indicate that there are other areas of potentially high C5 densities outside of the selected grid but within the Bay. Murison & Gaskin (1989) also reported variation in C5 densities and distribution in the Bay of Fundy, with the densest aggregations (> 832 – 1070 m^{-3}) being associated with right whales.

In the Bay of Fundy, environmental characteristics such as tidal fronts, thermal stratification, and circulation (also note in Cape Cod Bay, Mingshun et al. 2007) most likely promote dense aggregations of C5 (Fish & Johnson 1937, Murison & Gaskin 1989). The northern and southern tows were usually conducted within an hour of each other and thus most likely represented the prevailing tidal phase. In addition, weekly tows conducted over 10 weeks in 2006 and 2007 showed no significant temporal differences in C5 densities. The environmental characteristics listed above, along with vertical stability throughout the water column and low topographic relief, may minimize deep water mixing of C5 in this region (Woodley & Gaskin 1996, Baumgartner et al.

2003b), thereby facilitating dense aggregations of C5 required by right whales. Although tidal shifts may have affected the accuracy of C5 density estimates, I feel confident that my sampling regime is representative of C5 proportions available to right whales on a given day.

Lipid content and composition of copepods

Calanoid copepods begin accumulating lipid reserves in early summer in preparation for overwintering periods of low food availability (Lee & Hirota 1973). By late summer and early fall, C5 lipid reserves are dominated by WE components and can reach values ranging from 75 % to 90 % WE (Kattner & Krause 1987, Kattner & Graeve 1989, Kattner & Krause 1989, Kattner & Hagen 1995). WE dominated copepod lipid composition in the Bay of Fundy, comprising 94 % of the total lipid present. Michaud & Taggart (2007) reported that WE content in copepods in the Grand Manan Basin reaches a maximum of 75 %, which is considerably lower than reported by this study and others from higher latitudes (Kattner & Krause 1987, Kattner & Graeve 1991, Kattner & Hagen 1995). Copepod lipid content and WE proportions typically decreases with decreasing latitude (Kattner 1989) however, this study reports WE values (~ 90 % WE) closer to those found in polar regions (Kattner & Krause 1987, Kattner & Graeve 1991, Kattner & Hagen 1995). There was no variation in lipid composition between sampling sites within or outside of our selected region, indicating that copepod lipid composition did not vary between the selected regions over the course of this study. Although C5 biomass differed significantly between tow sites within and outside of the grid, C5 lipid content and composition did not vary, indicating that the concentration of C5 available to right whales

in the Bay of Fundy is an important parameter influencing right whale foraging patterns. The variation observed in copepod lipid content (4.55 – 9.91 %) in 2006 and 2007 was most likely reflective of differential lipid accumulation rates among patches of C5 and does not reflect seasonal or annual differences in lipid content.

In the Bay of Fundy, C5 *C. finmarchicus* copepods contained primarily saturated and monounsaturated FA, being dominated by 14:0, 16:0, 16:1n-7, 18:1n-9, 20:1n-9 and 22:1n-11 components (Table 4). Polyunsaturated components (20:5n-3 and 22:6n-3) comprised the remainder of the FA composition. The FAlc components were in much smaller proportions, and primarily consisted of 16:0, 22:1n-11 and 22:1n-9 (Table 4). Other studies, primarily from northern latitudes, report similar C5 FA and FAlc composition (Kattner & Krause 1987, Kattner et al. 1989, Kattner & Krause 1989, Kattner & Graeve 1991, Kattner & Hagen 1995, Albers et al. 1996).

Validations of assimilation models

The estimated assimilation rates of right whales were constrained by the inherent assumptions and caveats of the models used. Lipid assimilation rates were estimated by comparing the amount of lipid being ingested to the amount of lipid being eliminated. The assimilation estimates were based on two models: the first, an ingestion model designed specifically to estimate right whale ingestion rates (Baumgartner & Mate 2003) and second, a defecation model based on an allometric relationship of daily mammalian fecal production rates (Blueweiss et al. 1978, Lavigne 1982). There is some variation associated with each parameter of the ingestion model, including gape size, swim speed, duration at depth and copepod concentration available. Individual variation associated

with each of these parameters may significantly affect assimilation estimates by either overestimating or underestimating ingestion rates. Although gape size, swimming speed and duration at depth are variables that will vary depending on the individual whale, values for these parameters were based on previous studies (Mayo et al. 2001, Baumgartner & Mate 2003) that represent average calculations based on right whale behavior. Copepod densities available to right whales are likely to vary considerably for all individuals across spatial and temporal scales (Murison & Gaskin 1989, Woodley & Gaskin 1996, Baumgartner & Mate 2003, Michaud & Taggart 2007). Thus ingestion rates were modeled using a mean C5 density value from Baumgartner and Mate (2003) that was determined using the most sophisticated copepod quantification method to date (Baumgartner & Mate 2003) and assumed to be sufficient to support right whale metabolic requirements.

To determine whether the assimilation estimates are reasonable, values presented here were compared with estimated daily energetic requirements of right whales (Kenney et al. 1986). Kenney et al. (1986) reported that in order to meet basal metabolic requirements, a 40000 kg right whale must ingest 600 – 1600 kg of zooplankton day^{-1}. These ingestion estimates were based on consumption rates of 1.5 – 4.0 % of body weight per day (Sergeant 1969, Lockyer 1981b). Baumgartner & Mate (2003) reported C5 densities that could meet energetic demands predicted by Kenney et al. (1986). This study, and others conducted in the Bay of Fundy (Murison & Gaskin 1989, Michaud & Taggart 2007), reported much lower C5 densities that, assuming a 12 h feeding day, would not meet the energetic requirements estimated by Kenney et al. (1986). The C5 densities reported in the present study and by Murison & Gaskin (1989) and Michaud &

Taggart (2007) are closer to those that would be required to meet the energetic requirements for bowhead whales (Lowry & Frost 1984), which were 0.17 – 0.19 % of body weight, at 68 - 76 kg day^{-1}. Assuming a 12 hr feeding day and minimum and maximum C5 densities (188 – 2613 m^{-3}) measured from the present study, daily C5 ingestion rates were estimated as 0.05 – 0.75 % of body weight at 21 – 300 kg day^{-1}. This ingestion estimate is considerably lower than the 4.0 % of body weight predicted for baleen whales by Lockyer (1981a). Baumgartner and Mate's (2003) density estimate of 7481 C5 m^{-3} as applied to the assimilation model suggests an ingestion rate of 2.2 % (862258 g C5 day^{-1}) and is within the range of estimated ingestion rates of 1.5 – 4.0 % body weight for baleen whales (Sergeant 1969, Lockyer 1981a).

Energetic consequences to recovery

The overall low lipid content and the relative absence of FAlc in right whale fecal material suggest that right whales are highly efficient at metabolizing the vast majority of the lipids present in their diet. The energetic models further support high lipid assimilation efficiencies of right whales. Applying Baumgartner and Mate's (2003) mean C5 density of 7481 m^{-3} to the assimilation model, right whale total assimilation efficiency is approximately 92 % (Table 6), and lipid assimilation efficiencies are much higher at 98 % (Table 6). Assimilation efficiencies in other marine mammals, such as the Northern fur seal (*Callorhinus ursinus*) (Fadely et al. 1990), are thought to be high at > 90 % (Lockyer 2007), however Lockyer (1981a) estimated an assimilation efficiency of 80% for baleen mammals. The increased assimilation efficiency observed in the current study may be reflective of increased ingestion rates, specifically for reproductive females,

associated with intensive spring and summertime feeding as seen in other baleen whales (Lockyer 1986). Several studies of other baleen species report that adult females require more daily energy than adult males (Sergeant 1963, Lockyer 1981b, Innes et al. 1986, Markussen et al. 1992), suggesting that females have to spend more time feeding in order to meet increased energetic requirements of reproduction (i.e. growth, pregnancy, and lactation). Metabolically, it appears as though right whales are efficiently metabolizing the majority of the lipid from their diet however the amount of lipid available (m^{-3}) may be a more important factor associated with whales meeting energetic requirements.

Right whales rely on predictable foraging areas such as the lower Bay of Fundy, therefore fluctuations in C5 densities and C5 energetic value may affect the success of this struggling North Atlantic population. Future research directed toward long term monitoring of zooplankton densities and distribution in the Bay of Fundy may provide insight to understanding and predicting right whale habitat use and reproductive success. This study validates assumptions of right whale metabolic capabilities, such that current energetic models assume right whales metabolize all of the lipid in their diet, however prior to this study, complete metabolism of zooplankton lipids has not been determined. This study confirms current energetic assumptions, which may have underestimated right whale energetic requirements, and therefore may have implications for management applications of foraging assumptions and emphasize the importance of monitoring food quality and availability in the Bay of Fundy.

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