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Stable isotope and fatty acid analyses of samples from entrapped narwhals (*Monodon monoceros*)

Les analyses des isotopes stables et des acides gras des échantillons de narvals (*Monodon monoceros*) piégés

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ABSTRACT

Narwhals (*Monodon monoceros*) are medium sized odontocetes that live exclusively in Arctic waters. They are considered the most vulnerable Arctic cetacean based on their limited distribution, specialized physiological adaptation, and restricted diet which together limit their ability to modify behaviour in the face of changing climate. As a result of changes in seasonal ice characteristics associated with climate change and the limited ability of narwhal for behavioural modification, ice entrapment events may become more prevalent. In November 2008, an ice entrapment event occurred off the coast of Bylot Island, Nunavut near the community of Pond Inlet. Hundreds of narwhals attempted to travel outside of the inlets and fjords in the area before becoming entrapped by thickening ice. As a result, a humane Inuit harvest of animals occurred before the whales drowned. In total, 250 skin and blubber samples were collected from over 600 harvested whales. The entrapment event provided a unique sample because the individuals were likely closely related and many were females trapped with their young, whereas typically samples obtained from Inuit subsistence hunts are biased towards males. A dietary study was initiated to determine if diet differed among age classes and between sexes, and to determine if diet could be utilized to elucidate social structure in narwhals. Skin samples were analyzed for stable isotopes of carbon and nitrogen, which provides information on foraging location and trophic level, and dietary fatty acids in blubber, were used to identify primary prey items. Non-parametric statistics identified differences in isotopic signatures among age classes of narwhals, but no difference between sexes. Principal component analysis of fatty acids resulting from dietary intake qualitatively assessed feeding ecology of narwhals and determined there were no dietary differences between sexes or among age classes; however, distinct feeding groups were evident and genetic work is underway to determine the relatedness among these groupings. Emaciation appeared to have no significant impact on the fatty acid or isotopic signatures of samples from the entrapment event compared to published results, although more work is required to validate these conclusions.

RÉSUMÉ

Les narvals (*Monodon monoceros*) sont des odontocètes moyens qui vivent exclusivement dans les eaux arctiques. Ils sont jugés être les cétacés arctiques les plus vulnérables en raison de leur distribution limitée, de leur adaptation physique particulière et de leur alimentation limitée qui, ensemble, limitent leur capacité de modifier leur comportement devant les changements climatiques. En raison des changements aux caractéristiques de la glace saisonnière liés aux changements climatiques et à la capacité limitée du narval de modifier son comportement, des événements d'emprisonnement par les glaces peuvent devenir plus courants. En novembre 2008, un événement d'emprisonnement par les glaces a eu lieu au large de l'île Bylot, au Nunavut près de la collectivité de Pond Inlet. Des centaines de narvals ont tenté de sortir des passages et des fjords de la région avant d'être emprisonnés par la glace épaississante. Par conséquent, une récolte inuite sans cruauté a eu lieu avant la noyade des baleines. En tout, 250 échantillons de peau et de petit lard ont été prélevés de plus de 600 baleines récoltées. L'événement d'emprisonnement a fourni un échantillon unique parce que les sujets étaient étroitement liés, dont de nombreuses femelles emprisonnées avec leurs petits, tandis que les échantillons habituels prélevés des chasses de subsistance des Inuits sont surtout mâles. On a lancé une étude sur l'alimentation afin de déterminer si cette dernière diffère d'un groupe d'âge à l'autre et d'un sexe à l'autre, et afin de déterminer si l'alimentation pourrait être utilisée pour mieux connaître la structure sociale des narvals. Les échantillons de peau ont été analysés pour des isotopes stables de carbone et de nitrogène, qui fournissent de l'information sur les aires d'alimentation et le niveau trophique, et les acides gras alimentaires du petit lard ont été utilisés pour déterminer les proies principales. Les statistiques non paramétriques ont établi des différences dans les signatures isotopiques entre les groupes d'âge des narvals, mais aucune différence entre les sexes. L'analyse de la composante principale des acides gras découlant de l'apport alimentaire a évalué de manière qualitative l'écologie d'alimentation des narvals et a établi qu'il n'y avait aucune différence entre les sexes ou les groupes d'âge; cependant, des groupes d'alimentation distincts étaient évidents et du travail en génétique est en cours pour établir les liens de parenté entre ces groupes. L'émaciation ne semble pas avoir eu une incidence importante sur les acides gras ou les signatures isotopiques des échantillons de l'événement d'emprisonnement comparativement aux résultats publiés, bien qu'il faille plus de travail pour valider ces observations.

INTRODUCTION

Narwhals (*Monodon monoceros*) are medium sized odoncetes that live exclusively in Arctic waters. The Baffin Bay population spends summer in the northern fjords and inlets around Baffin Island, Canada and West Greenland (Dietz and Heide-Jørgensen 1995; Heide-Jørgensen et al. 2002, 2003). In November, they migrate south to Baffin Bay and Davis Strait where they live in pack ice with as little as 5 % open water (Laidre and Heide-Jørgensen 2005a). Narwhals are one of the Arctic marine mammals most vulnerable to climate change due to their limited distribution, specialized physiological adaptation, and restricted diet which collectively hinder their ability to modify behaviour in the face of changing climate (Laidre et al. 2008; Williams et al. 2010). Narwhals have been reported to repeatedly follow the same migratory patterns and return to the same fjords and inlets despite previous stochastic ice formation resulting in ice entrapments (Heide-Jørgensen et al. 2002). Ice entrapment events are a concern for conservation of the species as a whole and sustainability of specific narwhal stocks that are hunted as a food source by the Canadian Inuit.

In November 2008, an ice entrapment event occurred off the south coast of Bylot Island, Nunavut close to the community of Pond Inlet (72º 53.15" N, 78º 02.592" W) (Fig. 1). Days before the entrapment, thousands of whales were seen by locals of Pond Inlet, NU, moving quickly through freezing seas before being trapped (B. Dunn, Fisheries and Oceans Canada (DFO), pers. comm.). After observing the narwhals attempting to travel out of the inlets and fjords, it became apparent to residents that ice had become too concentrated and the remaining narwhal would be unlikely to reach the offshore open water. When travel on the ice became possible, local residents discovered entrapped narwhals at breathing holes about 50 km from open water. The Mittimatalik (Pond Inlet) Hunters and Trappers' Organization (HTO) then notified DFO and Nunavut Wildlife Management Board (NWMB) staff who agreed with the community's request to conduct a humane harvest before these animals drowned.

Samples from narwhals harvested during an entrapment event provide a unique opportunity to assess diet in a potentially closely related population. Narwhal dietary studies are usually biased towards male narwhals hunted by Inuit; however, this study includes a large sample of females, allowing a comparison of diet between the sexes. Samples collected from the entrapment event were also used to investigate differences in diet among age classes of narwhals. To date, studies investigating diet in *M. monoceros* have primarily relied on stomach content analyses and have revealed narwhal diet consists primarily of Arctic cod (*Boreogadus saida*), Greenland halibut (*Reinharditius hippoglossoides*), and squid (*Gonatus fabricii*) (Finley and Gibb 1982; Heide-Jørgensen et al. 1994; Laidre and Heide-Jørgensen 2005b); however, further investigation is warranted as stomach content analyses only provide a snapshot of what the whale has recently ingested and cannot provide insight into longer-term diet trends. Furthermore, stomach contents may be biased because prey specimens are digested partially or completely at various rates depending on tissue characteristics (e.g., bone, scales, muscle) during the process of digestion and can be underrepresented in the analyses. As a result of these limitations, more recent investigations of diet have relied on chemical signatures, such as those from fatty acids and stable isotopes. Fatty acids are relatively unmodified from prey to predator (Iverson et al. 1995; Kirsch et al. 1998; Lea et al. 2002) and in predator tissues, the stable isotope ratios of nitrogen and carbon are directly related to the ratios found in their prey (Peterson and Fry 1987); thus, both techniques can provide information regarding diet in narwhals. Fatty acids and stable isotope signatures also change as a result of nutritional stress and thus, can potentially be used to monitor stress in organisms (Hobson and Welch 1992; Hobson et al. 1993; Waheed et al. 1998; Cherel et al. 2005; Fuller et al. 2005; Gaye-Siessegger et al. 2007; Kempster et al. 2007; Williams et al. 2007; Liu et al. 2009).

Figure 1. Location of the entrapment site in relation to the nearest community Pond Inlet, Nunavut, Canada.

Lipid profiling and stable isotope analyses were used to investigate dietary differences among groups of narwhals and to determine if signatures are indicative of stress. For the latter objective, we compared our results to those previously published from a healthy sample of narwhal to determine how emaciation may have impacted isotopic and fatty acid signatures.

MATERIALS AND METHODS

DFO sent Blair Dunn and Jack Orr to Pond Inlet to collect samples from the harvested narwhals. The Pond Inlet Hunter and Trappers Organization reported 629 narwhals harvested. Researchers identified age classes based primarily on body size, since heads and tail flukes had often been removed. In total, 68 individuals were identified as calves, 210 as juveniles and 288 as adults. Samples of blood, skin, and blubber were collected from 22 calves, 46 juveniles, 68 adults, and 114 narwhals of unknown age class, for a total of 250 samples. Sample collection was very difficult given the vast quantity of narwhals, the minimal daylight hours, and the freezing temperatures; therefore, samples were crudely collected with a hatchet and it was not always feasible to determine age class while samples were being collected.

Skin samples were genetically analyzed to determine sex and preliminary results showed that 193 of the samples were female and 44 were male, with 13 being unclear and requiring further testing. These results were in line with the Inuit knowledge reporting that only the large males

and females were able to make the deep, long dives to escape the entrapment, with juveniles, calves, and the females associated with them, being trapped.

Lipids were extracted using the Folch procedure (Folch et al. 1957) with modifications recommended by Budge et al. (2006). Lipids were extracted from a 0.5 g subsection of blubber, which was isolated from the larger tissue sample. Blubber extractions were conducted as deep as the sample allowed; however, this was often within 1 cm from the skin. Lipids were extracted with 2:1 chloroform-methanol containing 0.01 % butylated hydroxytoluene. Blubber was mashed and removed from the solution and 3.5 ml of sodium chloride was added. Production of fatty acid methyl esters (FAME) was conducted utilizing a dichloromethane / 0.01 % butylated hydroxytoluene and Hilditch (a mixture of sulfuric acid and dry methanol) solution that was heated for 1 hour at 100 °C. Hexane, distilled water and sodium sulfate were added in series and the FAME and hexane were placed under an evaporative nitrogen stream. FAME samples were analyzed using an Agilent Technologies 7890A GC system, which uses gas chromatography for separating and analyzing the individual fatty acids. Fatty acids were described using A:Bn-X, where A is the number of carbon atoms, B the number of double bonds and X the position of the double bond closest to the terminal methyl group (Iverson et al. 2004).

Not all fatty acids arise from diet; many are biosynthesized within the predator and therefore should not be considered when using fatty acids to elucidate diet (Iverson et al. 2004). As a result, only those fatty acids known to transfer from prey to predator were used in analysis (Iverson et al. 2004). There were 33 dietary fatty acids identified in this study. An arbitrary small number (0.00001) was added to individuals who were interpreted as having 0 % of a given fatty acid (Kenkel 2006), as this is often a result of the detection limit of the GC system. Percent fatty acid values were then divided by the mean of the sample and log transformed (Kenkel 2006). Principal component analysis, a qualitative analysis that reduces the dimensionality of the data to only a few uncorrelated dimensions that explain the majority of the variability in the data, was conducted on the covariance matrix (Jolliffe 2002) and used to assess similarities and differences in diet among groups of narwhals. All multivariate statistics were calculated using packages developed for and implemented in the R environment (www.r-project.org).

For stable isotope analysis an approximately 0.5 g section of skin was cut from each sample, rinsed with water, cut into small pieces, and placed in the freeze-drier for a minimum of 40 hours. Samples were shipped to the stable isotope lab at Windsor University. Skin samples were lipid extracted using a 1:1 chloroform / methanol agitation protocol prior to analysis. Analysis of similarity (ANOSIM) was used to determine if significant differences in the $\delta^{13}C$ and $\delta^{15}N$ isotopes existed among age groups of narwhals and between sexes.

RESULTS

Average fatty acid percentages of all the biosynthesized and dietary fatty acids identified in this sample population are listed in Table 1. Results are compared to fatty acid profiles of another sample of narwhals analyzed by Thiemann et al. (2008) (Table 1). Fifty percent of the variance in narwhal fatty acid profiles was explained by the first two principal components (PC 1: 35 %, PC 2: 15 %). Fatty acid profiles of adults, juveniles and calves were not distinguishable (Fig. 2A), nor were those of males and females (Fig. 2B). Despite no obvious difference between age classes or sexes, there are some tight clusters of individuals that display more similar fatty acid signatures than others. The loadings plot, which reveal the individual fatty acids that drive the position of narwhals on the score plot, demonstrate that five fatty acids contributed substantially to the PCA, namely C21:5n-3, C22:4n-3, C22:5n-6, C22:4n-6, and C20:3n-6 (Fig. 2C).

There was no significant difference between the $\delta^{13}C$ and $\delta^{15}N$ isotope signatures of male and female narwhals (ANOSIM: Global R = -0.090; p = 0.917) (Fig. 3A); however, there was a difference in the isotopic signature of calves compared to juveniles (ANOSIM: Global R = 0.534, p = 0.001) and adults (ANOSIM: Global R = 0.466, p = 0.001), with no difference between adults and juveniles (Global R = -0.034, p = 0.985) (Fig. 3B).

Table 1. Fatty acid composition and major lipid classes (mass % of total FA ± SE) of adult and juvenile blubber samples collected from the Pond Inlet ice entrapment event. Fatty acid signatures of 20 narwhals from Thiemann et al. (2008) are also listed for comparative purposes.

Fatty acid	Narwhal (Eclipse sound entrapped sample)	† Narwhal (Thiemann et al. 2008)
SAT		
12:0*	0.86 ± 0.03	0.86 ± 0.06
14:0	5.11 ± 0.06	5.17 ± 0.16
Iso15	2.95 ± 0.12	0.89 ± 0.07
15:0	0.40 ± 0.01	0.27 ± 0.01
Iso16	0.20 ± 0.01	0.39 ± 0.03
16:0	6.11 ± 0.08	6.88 ± 0.39
7Me16:0	0.27 ± 0.01	0.26 ± 0.01
Iso17	0.14 ± 0.00	0.18 ± 0.01
17:0	0.12 ± 0.00	0.08 ± 0.00
18:0	1.06 ± 0.02	0.84 ± 0.07
MUFA		
14:1n-9	0.47 ± 0.02	0.75 ± 0.08
14:1n-7	0.20 ± 0.00	0.73 ± 0.07
14:1n-5*	2.64 ± 0.05	1.89 ± 0.13
16:1n-11	1.63 ± 0.03	1.16 ± 0.24
16:1n-9	1.45 ± 0.04	1.71 ± 0.14
16:1n-7	25.57 ± 0.26	23.03 ± 0.69
17:1	0.02 ± 0.00	0.13 ± 0.03
18:1n-11*	4.18 ± 0.05	5.42 ± 0.18
18:1n-9	15.27 ± 0.17	18.31 ± 0.45
18:1n-7	2.74 ± 0.12	3.25 ± 0.11
18:1n-5	0.36 ± 0.02	0.42 ± 0.02
20:1n-11	3.50 ± 0.05	4.04 ± 0.15
20:1n-9	6.64 ± 0.11	8.07 ± 0.39
20:1n-7	0.79 ± 0.06	0.57 ± 0.04
22:1n-11	3.15 ± 0.06	4.12 ± 0.41
22:1n-9	0.73 ± 0.04	0.71 ± 0.08
22:1n-7	0.23 ± 0.01	0.10 ± 0.01
PUFA		
16:2n-4	0.49 ± 0.01	0.12 ± 0.02
16:3n-4	0.39 ± 0.02	0.16 ± 0.02
16:4n-1	0.13 ± 0.01	0.09 ± 0.01
18:2n-6	0.76 ± 0.01	1.04 ± 0.08
18:2n-4	0.13 ± 0.00	0.04 ± 0.01
18:3n-6	0.10 ± 0.00	0.10 ± 0.01
18:3n-4	0.23 ± 0.03	0.07 ± 0.01
18:3n-3	0.28 ± 0.00	0.25 ± 0.01
18:4n-3	0.24 ± 0.00	0.23 ± 0.02
18:4n-1	0.18 ± 0.00	0.11 ± 0.01

20:2n-6	0.21 ± 0.00	0.15 ± 0.01
20:3n-6	0.01 ± 0.00	0.06 ± 0.00
20:4n-6	0.33 ± 0.01	0.17 ± 0.01
20:4n-3	0.25 ± 0.01	0.21 ± 0.02
20:5n-3	1.85 ± 0.06	2.06 ± 0.20
21:5n-3	0.09 ± 0.00	0.08 ± 0.01
22:4n-6	0.05 ± 0.01	0.02 ± 0.00
22:5n-6	0.08 ± 0.02	0.02 ± 0.00
22:5n-3	0.03 ± 0.04	1.00 ± 0.15
22:6n-3	1.74 ± 0.06	2.10 ± 0.24
∑ SAT	**17.22 ± 0.34**	**15.82 ± 0.81**
∑ MUFA	**69.57 ± 1.09**	**74.41 ± 3.22**
∑ PUFA	**7.57 ± 0.29**	**8.08 ± 0.84**

† Narwhals from Jones Sound (n=10), Repulse Bay (n=7) and Pond Inlet (n=3) were grouped together.
* Fatty acids that are all or primarily from biosynthesis

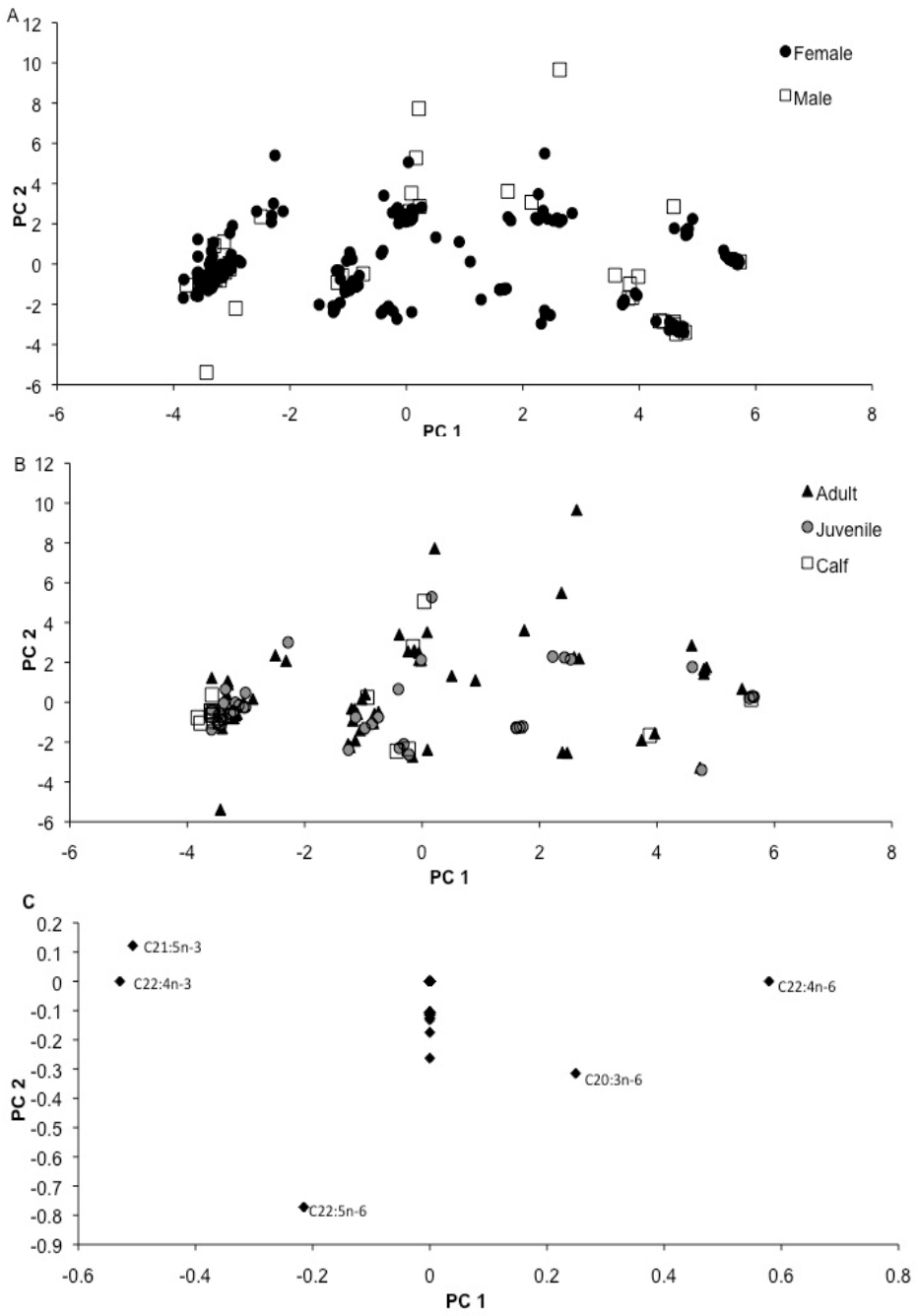

Figure 2. Principal component analysis (PCA) of the dietary fatty acids found in narwhal blubber (PC 1: 35 %, PC 2: 15 %). A) PCA score plot of female (circle) and male (square) narwhals. B) PCA score plot of narwhal adults (triangle), juveniles (circle), and calves (square). C) Factor loadings of individual fatty acids considered in the PCA. Only those five fatty acids that contributed substantially to the resulting PCA (those farthest from the origin) are labeled.

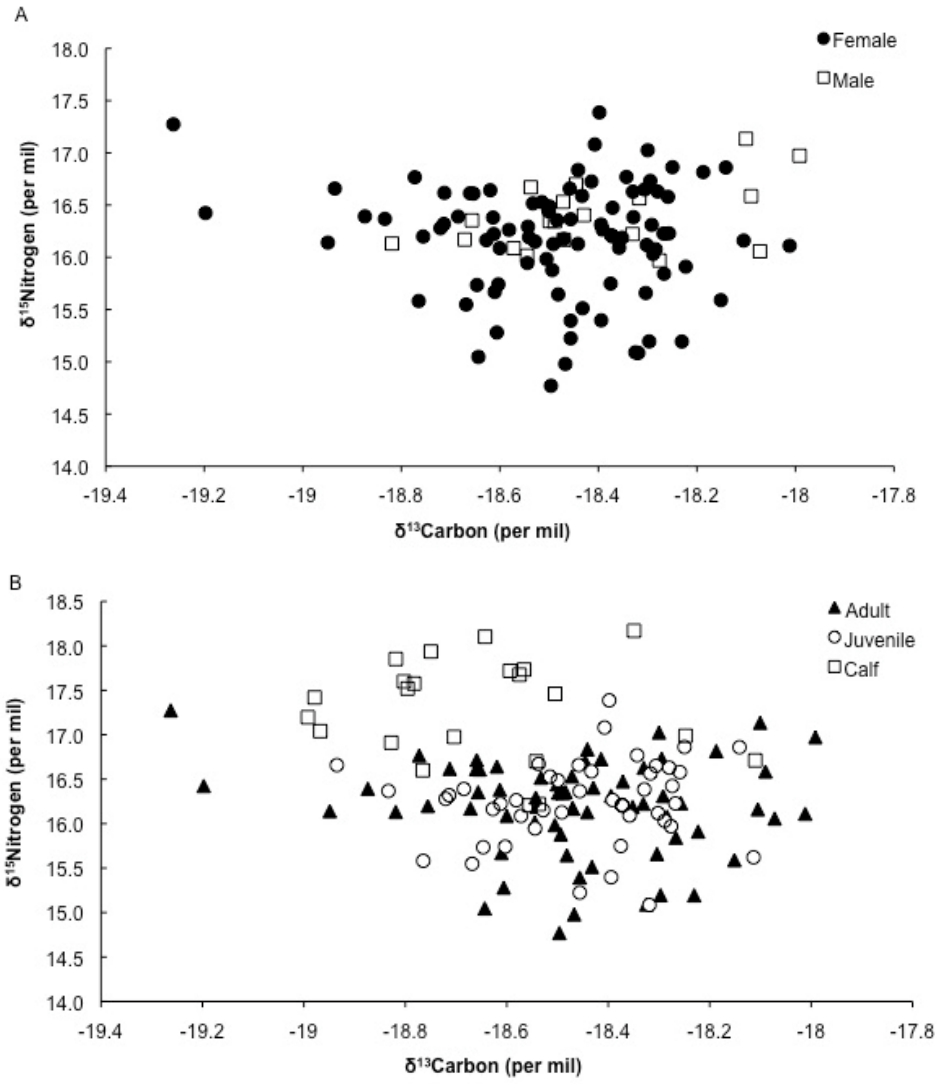

Figure 3. Stable isotope data plotted as $\delta^{13}C$ versus $\delta^{15}N$ for A) female (circle) and male (square) narwhals and B) narwhal adults (triangle), juveniles (circle), and calves (square).

DISCUSSION

This sample provides a unique opportunity to assess social structure in narwhals, as it is likely the entrapment captured many of the female clusters and their young that coexisted within the larger herd (Marcoux et al. 2009) and potentially captured entire familial lineages. Fatty acids did not differ among narwhal age classes; however, there did seem to be distinct groups of narwhal with similar fatty acid signatures and these groups consisted of a mixture of all age classes and sexes. Individuals with similar fatty acid signatures may forage and travel together and current genetic investigations are underway to determine if dietary signatures can help elucidate narwhal social structure by investigating whether individuals within the feeding groups are more closely related to each other than they are to the rest of the herd.

There were no differences in the stable isotope or fatty acid signatures between male and female narwhals, which supports previous findings that there is no dietary difference between narwhal sexes (Finley and Gibb 1982; Heide-Jørgensen et al. 1994). In order to determine what constitutes narwhal diet fatty acid signatures, potential prey items are being collected and will be compared to those obtained for narwhals. Stable isotopes, similar to fatty acids, can be used to quantify a predator's diet if the isotopic signatures of potential prey items are also known and are very different from one another (Crawford et al. 2008); thus, isotopic signatures of primary prey items are also being collected in order to determine diet utilizing isotope-mixing models. All of the fatty acids that contributed substantially to the resulting PCAs were polyunsaturated fatty acids suggesting that primary prey items may differ in their percentages of these particular fatty acids.

Unfortunately, histological testing of the skin and blubber samples collected after the humane harvest showed the narwhals became emaciated over a very short time span (within a few weeks) (Nielsen et al. 2010). Blubber appeared inflamed and red in color, which may have been a result of fat atrophy (Nielsen et al. 2010); thus, narwhals in this entrapment event may have begun metabolizing their fatty acid stores to compensate, which would result in changes to their fatty acid profiles. Fatty acids arise from biosynthesis, diet or a combination of the two (Iverson et al. 2004). Those fatty acids arising from diet or both biosynthesis and diet may differ between samples simply because of variable diets; therefore, only fatty acids that arise entirely from biosynthesis can be compared. C12:0, C18:1n-11, and C14:1n-5 all arise entirely from biosynthesis (Iverson et al. 2004). Biosynthesized fatty acids from the Pond Inlet entrapment event are comparable in terms of their mean percent to those obtained for a healthy narwhal sample investigated by Thiemann et al. (2008) (C12:0: 0.86 %, C18:1n-11: 2.64 %, and C14:1n-5: 4.18 % for the entrapped sample, and 0.86 %, 1.89 % and 5.42 % for the healthy population, respectively), with no obvious depletion in the emaciated narwhals.

One may expect that fatty acid synthesis would be reduced as a result of starvation; however, there may not have been enough time for nutritional stress to be exhibited in the fatty acid signature. Furthermore, because of logistical challenges with sample collection in the field (e.g., -40 ºC temperature and 24 hr darkness) it was not always feasible to sample the deepest blubber layers, which are mobilized first in times of stress (Koopman et al. 2002; Struntz et al. 2004). Biosynthesized fatty acids may not be selectively mobilized when narwhals are under stress and it is hard to draw any conclusions from only three fatty acids. Furthermore, without a healthy population for comparison, it is difficult to pinpoint the impacts nutritional stress has on fatty acid signatures. Current investigations from other individuals in the Eclipse Sound summering stock are underway and will be compared to signatures obtained from these animals to determine if differences exist between emaciated and healthy narwhals, and to establish which fatty acids are contributing to these differences.

Narwhal calves feed at a higher trophic level (indicated by a higher $\delta^{15}N$ signature) than adults and juveniles. Since calves are still dependent on their mother's milk and lactating mothers catabolize their own tissues to produce milk, nursing offspring often have isotope values higher than those exhibited by their mothers (Hobson and Sease 1998; Polischuk et al. 2001). $\delta^{13}C$ signatures, on the other hand, were significantly lower in narwhal calves. The same trend was observed in polar bear cubs, particularly when their mothers were in a period of fasting (Polischuk et al. 2001). Milk has high lipid content and lipids are carbon depleted relative to proteins; thus, this may result in calves having a lower $\delta^{13}C$ signature (Newsome et al. 2006). Periods of fasting could impact the degree of carbon depletion since lipid content within mother's milk may depend on their nutritional status and the status of their calves.

In general, animals that endure periods of fasting or nutritional stress are known to have differing stable isotope signatures during this time. δ^{15}N for instance may become enriched in organisms undergoing nutritional stress, as they are essentially feeding on their own tissues to survive the stress (Hobson et al. 1993; Cherel et al. 2005; Fuller et al. 2005; Gaye-Siessegger et al. 2007); however, this is not the case in all species and some studies have actually found nitrogen signatures to be depleted (Williams et al. 2007) or unaffected by nutritional stress (Kempster et al. 2007). The mean δ^{15}N signature for adult and juvenile narwhals was 16.20 ‰ ± 0.56 (standard deviation). Hobson and Welch (1992) found four narwhal muscle samples obtained from Admiralty Inlet (geographically close to the entrapment site (Fig. 1)) had mean δ^{15}N signatures of 15.8 ‰ ± 0.7; thus, the entrapped sample does not appear to have an enriched nitrogen signature compared to other values obtained from healthy narwhal samples. δ^{13}C signatures are relatively unpredictable with regard to nutritional stress, becoming enriched (Fuller et al. 2005; Gaye-Siessegger et al. 2007), depleted (Cherel et al. 2005; Williams et al. 2007), or unaffected (Hobson et al. 1993; Fuller et al. 2005; Kempster et al. 2007). The changes are primarily dependent upon the tissue being analyzed, the original condition of the animal enduring the stress, and the animal's natural response to nutritional stress including what tissue they are likely to mobilize first, which may be species and body size specific. Entrapped narwhals had a mean δ^{13}C signature of -18.47 ‰ ± 0.21 which was again comparable to signatures observed in healthy narwhal samples (-18.0 ‰ ± 0.4 (Hobson and Welch 1992)). Overall, it appears nutritional stress had negligible impact on isotopic signatures of narwhal collected for this investigation; however, future research will focus on comparing isotopic signatures in skin samples from the entrapped sample to those collected from healthy narwhals from the same Eclipse Sound summering stock

Ice entrapment events are a natural occurrence throughout the circumpolar Arctic; however, events of the magnitude seen in Pond Inlet may present a threat to the conservation of narwhals especially if they were to occur more frequently. If large entrapments became more common, this could affect sustainable harvest levels, which would be of concern for Inuit communities. Despite this, much knowledge can be gained from research into entrapment events and here we have provided preliminary results. Future investigations, including a comparative approach, may elucidate more clearly the impacts of emaciation on chemical signatures, and determine social foraging structure in narwhals, knowledge that is difficult to obtain in the wild.

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