



Chemical characterization of the oligosaccharides in Bryde's whale (*Balaenoptera edeni*) and Sei whale (*Balaenoptera borealis lesson*) milk

著者 (英)	Urashima Tadasu, Kobayashi Mami, Asakuma Sadaki, Uemura Yusuke, Arai Ikichi, Fukuda Kenji, Saito Tadao, Mogoe Toshihiro, Ishikawa Hajime, Fukui Yutaka
journal or publication title	Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology
volume	146
number	2
page range	153-159
year	2007-02
URL	http://id.nii.ac.jp/1588/00000497/

Chemical characterization of the oligosaccharides in Bryde's whale (*Balaenoptera edeni*) and Sei whale (*Balaenoptera borealis lesson*) milk

Tadasu Urashima¹, Mami Kobayashi², Sadaki Asakuma¹, Yusuke Uemura³, Ikichi Arai,² Kenji Fukuda¹, Tadao Saito⁴, Toshihiro Mogoe⁵, Hajime Ishikawa⁵ and Yutaka Fukui⁶

¹Graduate School of Food Hygiene, Obihiro University of Agriculture and Veterinary Medicine, Inada cho, Obihiro, Hokkaido, 080-8555, Japan.

²Department of Bioresource Chemistry, Obihiro University of Agriculture and Veterinary Medicine, Inada cho, Obihiro. Hokkaido, 080-8555. Japan

³Course of the Science of Bioresource, The United Graduate School of Agricultural Science, Iwate University, Morioka 020-8550, Japan.

⁴Department of Bio Production, Graduate School of Agriculture, Tohoku University, Tsutsumidori-Amamiya ,achi 1-1, Aoba-Ku, Sendai, 981-8555, Japan.

⁵The Institute of Cetacean Research, Toyomi-cho 4-18, Choh-Ku, Tokyo 104-0055, Japan.

⁶Laboratory of Animal Genetics and Reproduction, Obihiro University of Agriculture and Veterinary Medicine, Inada cho, Obihiro, Hokkaido, 080-8555. Japan.

Samples of milk from a Bryde's whale and a Sei whale contained 2.7 g/100 ml and 1.7 g/100 ml of hexose, respectively. Both contained lactose as the dominant saccharide along with small amounts of Neu5Ac(2-3)Gal(1-4)Glc (3'-N-acetylneuraminyllactose), Neu5Ac(2-6)Gal(1-4)Glc (6'-N-acetylneuraminyllactose) and Neu5Ac(2-6)Gal(1-4)GlcNAc(1-3)Gal(1-4)Glc (LST c). The dominance of lactose in the carbohydrate of these milks is similar to that of Minke whale milk and bottlenose dolphin colostrum, but the oligosaccharide patterns are different from those of these two species, illustrating the heterogeneity of milk oligosaccharides among the Cetacea.

Keywords: Bryde's whale, Sei whale, milk oligosaccharides, *Balaenoptera edeni*, *Balaenoptera borealis lesson*, Cetacea

1. Introduction

Previous studies have shown that, in general, aquatic mammals differ from terrestrial mammals in having relatively low level of carbohydrates and high level of fat in their milk (Pervaiz and Brew, 1986; Oftedal et al., 1988; Iverson et al., 1993; Lydersen and Kovacs, 1999). The milk of phocids contains very small amount of lactose along with many varieties of oligosaccharides (Urashima et al., 1997, 2001, 2003, 2004), while otariid milks contains neither lactose nor oligosaccharides. This suggests that suckling pinnipeds do not depend on lactose as an energy source, or depend on it to a very limited extent (Pilson and Kelly, 1962; Dosako et al., 1983; Urashima et al., 2004).

By contrast, the milk or colostrum of Cetaceans, including the Minke whale (*Balaenoptera acutorostrata*, Family Mysticeti) and bottlenose dolphin (*Tursiops truncatus*, Family Odontoceti) contains from 1 to 3% of carbohydrate of which lactose is the dominant component (Urashima et al., 2002; Shaw, 1971; Pervaiz and Brew, 1986; Uemura et al., 2005). This suggests that the calves of these cetaceans utilize lactose as well as lipids as an energy source. The milk/colostrum of Minke whale and bottlenose dolphin contains, in addition, small amounts of oligosaccharides (Urashima et al., 2002; Uemura et al., 2005). Although it is likely that these oligosaccharides function as anti-infection agents (Messer and Urashima, 2002), the chemical structures of the milk or colostrum oligosaccharides of these two species are very different.

There is as yet little published information on the milk carbohydrates of other Cetacean species. In this study, we have attempted to clarify the status of lactose and milk oligosaccharides in two closely related species of the sub-order Mysticeti, Bryde's whale and Sei whale, late in lactation. We also compare the structures of the oligosaccharides of these two species with those of the milk oligosaccharides of the Minke whale and the bottlenose dolphin.

2. Materials and Methods

2.1. Materials

The present study was performed in the Western North Pacific as part of the Japanese Whale Research Program under Special Permit (JARPN). The JARPN has been conducted by a non-profit research institution whose legal status is authorized

by the Government of Japan's Ministry of Agriculture, Forestry and Fisheries. The research is in full compliance with relevant international treaty, namely the International Convention for the Regulation of Whaling (ICRW). Sei and Bryde's whales captured for JARPN between May 2003 and August 2003 were used in this study. The whales were sampled in sub-areas 7, 8, 9, at 35 ° N and 140 ° E to 170 ° E, outside the economic exclusion zone (EEZ) of foreign countries as established by the International Whaling Commission (IWC). The whales were killed by explosive harpoons, which have been recognized by the IWC as the most humane method for killing whales, and is provided by schedule (Capture) of the International Convention for the Regulation of Whaling. Special efforts were made to reduce the time to death for all whales. Explosive harpoons were used as the primary killing method. A large caliber rifle was used as the secondary killing method when required. Body length and body weight were measured on board the vessel immediately after death. The body length and body weight of the female Sei and Bryde's whales were 14.82 meters and 22.75 tonnes, and 13.32 meters and 18.36 tonnes, respectively. To avoid death of the calves, the whales for this study were killed at the end of the normal lactation period.

During dissection of the whales, milk was obtained within 2 to 3 hours after death by massage of the mammary glands, care being taken to avoid contamination with water or blood. The milk samples were immediately frozen at -20 and stored at -80 until arrival at the laboratory.

2.2. Chemicals

Lactose monohydrate was purchased from Kanto Co., Tokyo, Japan. Neu5Ac(2-3)Gal(1-4)Glc (3'-N-acetylneuraminyllactose) and Neu5Ac(2-6)Gal(1-4)Glc (6'-N-acetylneuraminyllactose) were purchased from Sigma Co., St. Louis, MO, USA. Neu5Ac(2-6)Gal(1-4)GlcNAc(1-3)Gal(1-4)Glc (LST c) was obtained from Seikagaku Co., Tokyo, Japan.

2.3. Colorimetric assays

The carbohydrate concentrations of Bryde's and Sei whale milk were assayed as total hexose using the phenol - H₂SO₄ method (Hodge and Hofreiter, 1962). Lactose

was used as the standard. The sialic acid content was determined by the periodate – resorcinol method using N-acetylneuraminic acid as the standard (Jourdian et al., 1971).

2.4. Isolation of oligosaccharides from whale milk

The milk (20 mL) from each whale was thawed, diluted with 4 volumes of water and then extracted with four volumes of chloroform/methanol 2:1 (v/v). The emulsions were centrifuged at 4 and 4000 xg for 30 min. The lower chloroform layer and the denatured protein were discarded. The methanol was removed from the upper layer by rotary evaporation, and the residues were freeze-dried. The resulting white powder was designated as the ‘carbohydrate fraction’.

The carbohydrate fraction from each milk sample was separately dissolved in 2 mL of water and the solution passed through a Bio gel P-2 (< 45 μm) column (2.5 X 100 cm), which had been calibrated with galactose (monosaccharide), lactose (disaccharide) and raffinose (trisaccharide) (2 mg of each) at room temperature (chromatograms in Fig. 1). Elution was done with water at a flow rate of 15 mL/h and 5 mL fractions were collected. Aliquots (0.5 mL) of each fraction were analyzed for hexose and sialic acid. Peak fractions were pooled and freeze-dried. Components in the peak fractions from both milks, referred to BW 5 and SW 2 (see Fig. 1a and 1b), were analyzed by ¹H-NMR spectroscopy.

The components in BW 1 from the Bryde’s whale milk and in SW 1 from the Sei milk (see Fig. 1a and 1b) were each dissolved in 2 mL of 50 mM Tris – hydroxyaminomethane – HCl buffer (pH 8.7) and subjected to anion exchange chromatography on DEAE – Sephadex A-50 column (1.5 X 35 cm) equilibrated with the same buffer (chromatograms in Fig. 1c and 1d). The unadsorbed components were eluted with 150 mL of the buffer. Elution was done at a flow rate of 15 mL/h and fractions of 5 mL were collected. Aliquots (0.5 mL) of each fraction were analyzed for hexose. Peak fractions, again designated as BW 1 and SW 1 (see Fig. 1c and 1d) were pooled and freeze-dried and the product dissolved in 2 mL of water and passed through a Bio Gel P-2 column under the same conditions as described above. Peak fractions (elution volume: 150 ~ 190 mL) that contained hexose, as indicated by the phenol – H₂SO₄ method, were pooled and freeze – dried.

The components in BW 1 and SW 1 (see Fig. 1c and 1d) during anion exchange

chromatography and successive gel chromatography on Bio Gel P-2 were further separated by high performance liquid chromatography (HPLC) (chromatograms in Fig. 2). The HPLC was performed using a SHIMADZU LC-10AT pump with a TSK gel Amido – 80 column (4.6 X 250 mm, pore size 80 Å, particle size 5 µm, Tosoh Co., Tokyo, Japan). The mobile phase was 50 and 80% (v/v) acetonitrile (CH₃CN) in 15 mM potassium phosphate buffer (pH 5.2). Elution was done with a linear gradient of CH₃CN from 80 to 50% at 40 min at a flow rate of 1 mL/min. Eluted materials were detected by measuring the absorbance at 195 nm. The peak fractions of oligosaccharides were pooled and lyophilized.

2.5. ¹H-NMR spectroscopy

¹N-NMR spectra were recorded in D₂O (100.00 atom%D, Aldrich, Milwaukee, WI, USA) at 500 MHz with a Jeol ECP-500 FT-NMR spectrometer or at 600 MHz with a Varian INOVA 600 spectrometer, operated at 293.1 K. Chemical shifts are expressed in ppm down-field from internal 3-(trimethylsilyl)-1-propane sulfonic acid, sodium salt (TPS), but were actually measured by reference to internal acetone (δ = 2.225).

3. Results

The Bryde's whale milk contained 2.7 g/100 mL hexose and 0.26 g/100 mL sialic acid, whereas the Sei whale milk contained 1.7 g/100 mL hexose and 0.15 g/100 mL sialic acid. During chromatography on Bio Gel P-2, the carbohydrate fractions of these samples each separated into several peaks with one very large peak and a few smaller peaks that were eluted prior to the large peak, as shown in Figs. 1a and 1b. It was assumed that only the components in the smaller peaks in both chromatograms contained sialyl oligosaccharides, because only the contents of these peaks gave a positive result with periodate – resorcinol (Fig. 1a and 1b).

The components in the large peaks BW 5 and SW 2 had the same R_f value as lactose during TLC, suggesting that these were lactose. This was confirmed by ¹H-NMR spectroscopy (see below). During TLC, no spots were detected with peaks BW 2, BW 3 and BW 4; the components in these fractions were therefore not characterized in this study.

BW 5

The $^1\text{H-NMR}$ (chemical shifts in Table 1) of BW 5 had the anomeric resonances of reducing β -Glc and α -Glc, and (1-4) linked Gal at 5.223, 4.665 and 4.451, respectively. As the $^1\text{H-NMR}$ pattern was essentially to the same as that of lactose, BW 5 was characterized as Gal(1-4)Glc.

SW 2

The $^1\text{H-NMR}$ (chemical shifts in Table 2) of SW 2 had the anomeric resonances of reducing β -Glc and α -Glc, and (1-4) linked Gal at 5.222, 4.665 and 4.448, respectively. As the $^1\text{H-NMR}$ pattern was essentially to the same as that of lactose, SW 2 was characterized as Gal(1-4)Glc.

The components in BW 1 and SW 1 separated from the Bryde's whale and Sei whale milks, respectively, were further subjected to anion exchange chromatography. The resulting peaks were again designated as BW 1 and SW 1 (Fig. 1c and 1d), whose contents were each subjected to HPLC (Fig. 2). The contents of the resulting peaks designated as BW1-1, BW1-2, BW1-3 (Fig. 2a) and, SW1-1, SW1-2 and SW1-5 (Fig. 2b) were characterized by $^1\text{H-NMR}$.

BW1-1

The $^1\text{H-NMR}$ spectrum (chemical shifts in Table 1) of BW1-1 was essentially the same as that of authentic 3'-N-acetylneuraminyllactose; it was characterized to be Neu5Ac(2-3)Gal(1-4)Glc.

The spectrum had characteristic H-3 axial, H-3 equatorial and NAc resonances at 1.798, 2.756 and 2.030, respectively, and H-3 of (1-4) linked Gal, which was substituted by a Neu5Ac residue at OH-3, at 4.113 showing the presence of a Neu5Ac(2-3)Gal unit. The spectrum had the H-1 shifts of β -Glc, α -Glc and (1-4) linked Gal at 5.221, 4.663 and 4.531, respectively.

BW1-2

The $^1\text{H-NMR}$ spectrum (chemical shifts in Table 1) of BW1-2 was essentially the same as that of authentic 6'-N-acetylneuraminyllactose; it was characterized to be Neu5Ac(2-6)Gal(1-4)Glc.

The spectrum had the anomeric shifts of α -Glc, β -Glc and (1-4) linked Gal at 5.225, 4.669 and 4.428, respectively, and H-3 axial, H-3 equatorial and NAc shifts of (2-6) linked Neu5Ac at 2.712, 1.743 and 2.029, respectively.

BW1-3

Because the $^1\text{H-NMR}$ spectrum (chemical shifts in Table 1) of BW1-3 was essentially the same as that of authentic LST c, it was characterized to be Neu5Ac(2-6)Gal(1-4)GlcNAc(1-3)Gal(1-4)Glc.

The spectrum had the characteristic H-3 axial, H-3 equatorial and NAc resonances of Neu5Ac residue at 1.723, 2.668 and 2.027, respectively, showing the presence of a Neu5Ac(2-6)Gal unit. The spectrum had H-1 shifts of α -Glc, β -Glc, (1-3) linked GlcNAc and two (1-4) linked Gal at 5.221, 4.665, 4.729, 4.455 and 4.443, respectively, and H-4 shift of (1-4) linked Gal, which was substituted at OH-3 by β linked GlcNAc, at 4.160.

SW1-1

The $^1\text{H-NMR}$ spectrum (chemical shifts in Table 2) of SW1-1 was essentially the same as that of authentic 3'-N-acetylneuraminyllactose; it was characterized to be Neu5Ac(2-3)Gal(1-4)Glc.

The spectrum had the characteristic H-3 axial, H-3 equatorial and NAc residues at 1.794, 2.750 and 2.027, respectively, and H-3 of (1-4) linked Gal, which was substituted by a Neu5Ac residue at OH-3, at 4.111 showing the presence of a Neu5Ac(2-3)Gal unit. The spectrum had the H-1 shifts of α -Glc, β -Glc and (1-4) linked Gal at 5.220, 4.660 and 4.526, respectively.

SW1-2

The $^1\text{H-NMR}$ spectrum (chemical shifts in Table 2) of SW1-2 was essentially the same as that of authentic 6'-N-actylneuraminylactose; it was characterized to be Neu5Ac(2-6)Gal(1-4)Glc.

The spectrum had the anomeric shifts of α -Glc, β -Glc and (1-4) linked Gal at 5.224, 4.668 and 4.426, respectively, and H-3 axial, H-3 equatorial and NAc shifts of (2-6) linked Neu5Ac at 2.709, 1.741 and 2.026, respectively.

SW1-5

Because the $^1\text{H-NMR}$ spectrum (chemical shifts in Table 2) of SW1-5 was essentially the same as that of authentic LST c, it was characterized to be Neu5Ac(2-6)Gal(1-4)GlcNAc(1-3)Gal(1-4)Glc.

The spectrum had the characteristic H-3 axial, H-3 equatorial and NAc resonances of Neu5Ac residue at 1.722, 2.670 and 2.028, respectively, showing the presence of a Neu5Ac(2-6)Gal unit. The spectrum had H-1 shifts of α -Glc, β -Glc, (1-3) linked GlcNAc and two (1-4) linked Gal at 5.221, 4.665, 4.730, 4.456 and 4.442, respectively, and H-4 shift of (1-4) linked Gal, which was substituted at OH-3 by linked GlcNAc, at 4.158.

4. Discussion

The milk or colostrum of aquatic mammals contains only 1 to 3% of carbohydrate (Urashima et al., 2001a, 2002, 2003, 2004; Uemura et al., 2005; Shaw, 1971; Pervaiz and Brew, 1986). Phocid milks contain higher concentrations of oligosaccharides than of lactose (Urashima et al., 2001a, 2003, 2004), whereas in bottlenose dolphin milk and colostrum and a sample of Minke whale milk lactose is the dominant saccharide (Uemura et al., 2005; Urashima et al., 2002). In the present investigation, Bryde's whale and Sei whale milks were found to contain 2.7 g/100 mL and 1.7 g/100 mL of carbohydrate, respectively, of which lactose was the largest component. This suggests that their calves utilize lactose as an energy source.

This situation resembles that found in the bottlenose dolphin and the Minke whale (Uemura et al., 2005; Urashima et al., 2002). However, the milk of a beluga at late

lactation and of Stejneger's beaked whale collected at 20 ~ 40 days post partum contained only little or no lactose (Urashima et al., 2002; Ulley et al., 1984). In addition, a different milk sample of Minke whale contained more oligosaccharide than lactose, but it is possible that this whale had almost stopped lactating, bearing in mind the fact that milk composition at late lactation is often known to be a typical (Urashima et al., 2002). Pervaiz and Brew (1986) reported that Atlantic bottlenose dolphin milk collected at 198 ~ 210 days post partum contained 2.5% of lactose. Jenness et al. (1974) reported that the mature milks of the following Cetacea species contained from 0.7 to 1.3% of carbohydrate, although accurate values for lactose content were unknown; beluga, Atlantic bottlenose dolphin, spotted dolphin, spotted porpoise, spinner porpoise, Atlantic harbor porpoise, blue whale, finback whale and humpback whale. It was also reported that late lactation milks of the blue whale and fin whale contained 1.3 and 2.3% of lactose and other sugars (White, 1953; Gregory et al., 1955; Ohta et al., 1955). These differences regarding the dominance or non – dominance of lactose in the milk carbohydrate fraction of Cetacea may be due to differences in the time of lactation at which the milks were collected, as well as reflect species specificity among the Cetacea. The Bryde's whale and Sei whale milks were collected late in lactation. Thus, the data concerning lactose and milk oligosaccharides in Cetacea milk are limited and, in addition, there is little information concerning the stages at which the milk samples were collected.

The ratio of milk oligosaccharides to lactose varies among mammalian species. The concentration of milk oligosaccharides is greater than that of lactose in the milk of monotremes and marsupials. As the gestation period is short in these species, they have altricial neonates and, it has been suggested that their high ratio of milk oligosaccharides to lactose in the milks may relate to this altricity (Messer and Urashima, 2002). In eutherians, the milk of the Canioidea, including bears (Urashima et al., 2000), seals (Urashima et al., 2001a, 2003, 2004), whitenosed coati (Urashima et al., 1999) and mink (Urashima et al., 2005), other than dog (Bubb et al., 1999) of Carnivora, exceptionally contain more milk oligosaccharides than lactose. Human (Newburg and Neubauer, 1995) and elephant (Uemura et al., in press) milks, in addition, contain relatively large amounts of milk oligosaccharides as well as a great variety, although the dominant saccharide in these milks is lactose. Human milk contains more than 100 kinds of milk oligosaccharides (Newburg and Neubauer, 1995). In these eutherian

species, milk oligosaccharides may be very significant for infants, playing biological roles such as of prebiotics which stimulate beneficial microorganisms in the infant colon and of receptor analogues which inhibit the attachment of pathogenic microorganisms to the colonis mucosa (Messer and Urashima, 2002). It has also been suggested that small amounts of human milk oligosaccharides are absorbed in the infant small intestine and that these have immuno modulation effects within the circulation (Bode, 2006). On the other hand, the milk of most eutherians' contains only small amounts of oligosaccharides whose variety is small. For example, bovine mature milk contains only trace amounts of milk oligosaccharides (Gopal and Gill, 2000). In the milks of Bryde's whale and Sei whale, the ratio of milk oligosaccharides to lactose is also very low and the milk oligosaccharides pattern is very simple, a situation which is rather similar to that of cows milk. Therefore milk oligosaccharides are probably not very significant for the neonates of these species. It would be desirable, in further studies, to clarify why the physiological demand for milk oligosaccharides is different among these mammalian species. It is notable, however, that bovine colostrum contains more than 1 g/L of milk oligosaccharides (Nakamura et al., 2003), this suggests that newborn calves require milk oligosaccharides, whose significance decreases in the transitional and mature cows milk.

The comparison of milk oligosaccharides within Cetacea, including Bryde's whale, Sei whale, bottlenose dolphin and Minke whale is shown in Fig. 3. This comparison is restricted to species of Cetacea in this paper. Please refer to Urashima et al. (2001b) for a comparison of the milk oligosaccharides of other mammalian species. 3'-N-Acetylneuraminyllactose and 6'-N-acetylneuraminyllactose, found in Bryde's whale and Sei whale milk, were also identified in bottlenose dolphin colostrum (Uemura et al., 2005), whereas they were absent from Minke whale milk. LST c (Urashima et al., 2002), which was identified in Bryde's and Sei whale milk, was also found in Minke whale (Urashima et al., 2002), but this was not identified in bottlenose dolphin colostrum (Uemura et al., 2005). Neu5Ac(2-3)[GalNAc(1-4)]Gal(1-4)Glc (GM₂ tetrasaccharide), which was found in bottlenose dolphin colostrum (Uemura et al., 2005), was not identified in Bryde's whale, Sei whale and Minke whale milk. The dominance of the sialyl oligosaccharides containing Neu5Ac(2-3) residue than those containing Neu5Ac(2-6) residue was in common in these Cetacea species milk/colostrum.

Fuc(1-2)Gal(1-4)Glc (2'-fucosyllactose) and GalNAc(1-3)[Fuc(1-2)]Gal(1-4)Glc (A-tetrasaccharide), which were identified in Minke whale milk (Urashima et al., 2002), was not found in Bryde's whale and Sei whale milk. Gal(1-4)Gal(1-4)Glc (globotriose), which was identified in bottlenose dolphin colostrum (Uemura et al., 2005), was not found in these milks. These observations suggest a large degree of heterogeneity in milk oligosaccharides among cetacean species. Further studies with the milk of other Cetaceans and of milk collected earlier in lactation may shed light on the reasons for this heterogeneity. However, our finding that the ratio of lactose to oligosaccharides, and the structures of the oligosaccharides, were similar in Bryde's and Sei whales is most likely related to the close phylogenetic relationship between these two species within the suborder Mysticeti.

The small amounts of milk oligosaccharides in Bryde's and Sei whale milks may function as receptor analogues, inhibiting the attachment of pathogenic bacteria and viruses to their calves' colonocytes, as is believed to be the case in human infants (Newburg and Neubauer, 1995).

Acknowledgements

The authors thank the captain and crew of the research ship, *Nisshinn-maru* for collecting milk from Sei and Bryde's whales . This study was partially supported by a Grant from the 21st Century COE Program (A-1), Ministry of Education, Culture, Sports, Science and Technology, Japan, and a Grant-in-Aid (Research and Development Program for New Bio-industry Initiatives) from Bio-oriented Technology Research Advancement Institution of the National Agriculture and Food Research Organization, Japan.

References

- Bode,L. 2006. Recent advances on structure, metabolism, and function of human milk oligosaccharides. J. Nutr. 136, 2127-2130.
- Bubb, W.A., Urashima, T., Kohso, K., Nakamura, T., Arai, I., Saito, T. 1999. Occurrence of an unusual lactose sulfate in dog milk. Carbohydr. Res. 318, 123-128.

Dosako, S., Tanaya, S., Kimura, T., Ohmori, T., Daikoku, H., Suzuki, N., Sawa, J., Kano, K., Katayama, S. 1983. Milk of northern fur seal: composition, especially carbohydrate and protein. *J. Dairy Sci.* 66, 2076-2083.

Gopal, P.K., Gill, H.S. 2000. Oligosaccharides and glycoconjugates in bovine milk and colostrum. *Br. J. Nutr.* 84, Suppl 1, S69-S74.

Gregory, M.E., Kon, S.K., Rowland, S.J., Thompson, S.Y. 1955. The composition of the milk of the blue whale. *J. Dairy Res.* 22, 108-112.

Hodge, J.E., Hofreiter, B.T., 1962. Determination of reducing sugars and carbohydrates. *Methods Carbohydr. Chem.* 1, 380-394.

Iverson, S.J., Bowen, W.D., Boness, D.J., Oftedal, O.T. 1993. The effect of maternal size and milk energy output on pup growth in grey seals (*Halichoerus grypus*). *Physiol. Zool.* 66, 61-88.

Jenness, R. 1974. The composition of milk. In "Lactation": A Comprehensive Treatise" (B.L. Larson and V. R. Smith, eds.), Vol. , pp. 3-107, Academic Press, New York.

Jourdain, G.W., Dean, L., Roseman, S., 1971. The sialic acids . A periodate-resorcinol method for the quantitative estimation of free sialic acid and their glycosides. *J. Biol. Chem.* 246, 430-435.

Lydersen, C., Kovacs, K.M., 1999. Behaviour and energetics of ice – breeding, North Atlantic phocid seal during the lactation period. *Mar. Ecol. Prog. Ser.* 187, 265-281.

Messer, M., Urashima, T. 2002. Evolution of milk oligosaccharides and lactose. *Trends Glycosci. Glycotech.* 14, 153-176.

Nakamura, T., Kawase, H., Kimura, K., Watanabe, Y., Ohtani, M., Arai, I., Urashima, T. 2003. Concentrations of sialyloligosaccharides in bovine colostrum and milk during the

prepartum and early lactation. J. Dairy Sci. 86, 1315-1320.

Newburg, D.S., Neubauer, S.H. 1995. Carbohydrates in milks: Analysis, Quantities, and Significance. In Handbook of Milk Composition, pp. 273-349 (R.G. Jensen ed.) San Diego, Academic Press.

Oftedal, O.T., Boness, D.J., Bowen, W.D. 1988. The composition of hooded seal (*Cystophora cristata*) milk: an adaptation for postnatal fattening. Can J. Zool. 66, 318-322.

Ohta, K., Watarai, T., Oishi, T. 1955. Composition of fin whale milk. Sci. Rep. Whales Res. Inst. 10, 151-167.

Pervaiz, S., Brew, K., 1986. Compositions of the milks of the bottlenose dolphin (*Tursiops truncatus*) and the Florida manatee (*Trichechus manatus latirostris*). Comp. Biochem. Physiol. A84, 357-360.

Pilson, M.E.Q., Kelly, A.L. 1962. Composition of the milk from *Zalophus californianus*, the California sea lion. Science 135, 104-105.

Shaw, D.H. 1971. Neutral carbohydrates in the milk of the bottlenose dolphin (*Tursiops truncatus*). Carbohydr. Res. 19, 419-422.

Uemura, Y., Asakuma, S., Nakamura, T., Arai, I., Taki, M., Urashima, T. 2005. Occurrence of a unique sialyl tetrasaccharide in colostrum of a bottlenose dolphin (*Tursiops truncatus*). Biochim. Biophys. Acta 1725, 290-297.

Uemura, Y., Asakuma, S., Yon, L., Saito, T., Fukudam K., Arai, I., Urashima, T. in press. Structural determination of the oligosaccharides in the milk of an Asian elephant (*Elephas maximus*). Comp. Biochem. Physiol.

Ulley, D.E., Schwartz, C.C., Whetter, P.A., Rajeshwar Rao, T., Euber, J.R., Cheng, S.G., Brunner, J.R. 1984. Blue-green color and composition of Stjerner's beaked whale

(*Mesoplodon stejnegeri*) milk. Comp. Biochem. Physiol. B79, 349-352.

Urashima, T., Hiramatsu, Y., Murata, S., Nakamura, T., Messer, M. 1997. Identification of 2'-fucosyllactose in milk of the crabeater seal (*Lobodon carcinophagus*). Comp. Biochem. Physiol. B116, 311-314.

Urashima, T., Yamamoto, M., Nakamura, T., Arai, I., Saito, T., Namiki, M., Yamaoka, K., Kawahara, K. 1999. Chemical characterization of the oligosaccharides in a sample of milk of a white-nosed coati, *Nasua narica* (Procyonidae: Carnivora). Comp. Biochem. Physiol. A123, 187-193.

Urashima, T., Yamashita, T., Nakamura, T., Arai, I., Saito, T., Derocher, A.E., Wiig, O. 2000. Chemical characterization of milk oligosaccharides of the polar bear, *Ursus maritimus*. Biochim, Biophys. Acta 1475, 395-408.

Urashima, T., Arita, M., Yoshida, M., Nakamura, T., Arai, I., Saito, T., Arnould, J.P.Y., Kovacs, K.M., Lydersen, C. 2001a. Chemical characterization of the oligosaccharides in hooded seal (*Cystophora cristata*) and Australian fur seal (*Arctocephalus pusillus doriferus*) milk. Comp. Biochem. Physiol. B128, 307-323.

Urashima, T., Saito, T., Nakamura, T., Messer, M. 2001b. Oligosaccharides of milk and colostrum in non-human mammals. Glycoconj. J. 18, 357-371.

Urashima, T., Sato, H., Munakata, J., Nakamura, T., Arai, I., Saito, T., Tetsuka, M., Fukui, Y., Ishikawa, H., Lydersen, C., Kovacs, K.M. 2002. Chemical characterization of the oligosaccharides in beluga (*Delphinapterus leucas*) and Minke whale (*Balaenoptera acutorostrata*) milk. Comp. Biochem. Physiol. B132, 611-624.

Urashima, T., Nakamura, T., Yamaguchi, K., Munakata, J., Arai, I., Saito, T., Lydersen, C., Kovacs, K.M. 2003. Chemical characterization of the oligosaccharides in high Arctic harbour seal (*Phoca vitulina vitulina*) milk. Comp. Biochem. Physiol. A135, 549-563.

Urashima, T., Nakamura, T., Nakagawa, D., Noda, M., Arai, I., Saito, T., Lydersen, C., Kovacs, K.M. 2004. Characterization of oligosaccharides in milk of bearded seal (*Erignathus barbatus*). *Comp. Biochem. Physiol.* B138, 1-18.

Urashima, T., Nakamura, T., Ikeda, A., Asakuma, S., Arai, I., Saito, T., Oftedal, O.T. 2005. Characterization of oligosaccharides in milk of a mink, *Mustela vison*. *Comp. Biochem. Physiol.* A142, 461-471.

White, J.C.D. 1953. Composition of whales' milk. *Nature* 171, 612.

Fig. 1 Separation of the carbohydrate fractions from the milks of Bryde's whale and sei whale. Gel chromatograms of the carbohydrate fractions from the milks of Bryde's whale (a) and Sei whale (b) on a Bio Gel P-2 column (2.6 X 100 cm). Each fraction was monitored for hexose by the phenol – H₂SO₄ method (—) and for sialic acid by the periodate – resorcinol method (· ·). Anion exchange chromatograms of BW-1 (c) and SW-1 (d) separated from Bryde's and Sei whale milk, respectively, by the gel chromatography. The fractions were monitored by the phenol – H₂SO₄ method.

Fig. 2 HPLC of the carbohydrate fractions BW-1 and SW-1 separated from Bryde's and Sei whale milk. HPLC was done using a Shimadzu LC-10ATVP pump on a TSK – gel Amido – 80 column (4.6 X 250 cm, pore size 80 Å, particle size 5 mm). The mobile phase was 50 and 80% acetonitrile (CH₃CN) in 50 mM potassium phosphate buffer. Elution was done using a linear gradient of CH₃CN from 80 to 50% at 40 °C at a flow rate of 1 mL/min. Peaks were detected by UV absorption.

Fig. 3 Comparison of milk oligosaccharides of Bryde's whale, Sei whale, Minke whale and bottlenose dolphin

Table 1. ¹H-NMR chemical shifts of the oligosaccharides separated from Bryde's whale milk

Reporter group	Residue	Chemical shifts, δ (coupling constants, Hz)			
		BW-1-1	BW-1-2	BW-1-3	BW-5
H-1	Glc α	5.221 (3.9)	5.225 (3.9)	5.221 (3.8)	5.223 (3.8)
	Glc β	4.663 (7.8)	4.669 (8.3)	4.665 (8.2)	4.665 (8.0)
	Gal'(β 1-4)	4.531 (7.8)	4.428 (7.8)	4.443 (7.0)	4.450 (8.0)
	Gal''(β 1-4)	-	-	4.455 (7.6)	-
	GlcNAc(β 1-3)	-	-	4.729 (7.6)	-
H-3ax	Neu5Ac(α 2-3)	1.798 (12.2 ^a , -12.2 ^b)	-	-	-
	Neu5Ac(α 2-6)	-	1.743 (12.2 ^a , -12.2 ^b)	1.723 (12.0 ^a , -12.3 ^b)	-
H-3eq	Neu5Ac(α 2-3)	2.756 (4.9 ^c)	-	-	-
	Neu5Ac(α 2-6)	-	2.712 (4.9 ^c)	2.668 (4.7 ^c)	-
H-3	Gal'(β 1-4)	4.113 (2.4 ^d)	-	-	-
H-4	Gal'(β 1-4)	-	-	4.160 (2.8 ^e)	-
NAc	GlcNAc(β 1-3)	-	-	2.053	-
	Neu5Ac(α 2-3)	2.030	-	-	-
	Neu5Ac(α 2-6)	-	2.029	2.027	-

^aJ_{ax, 4}; ^bJ_{3ax, 3eq}; ^cJ_{3eq, 4}; ^dJ_{3, 4}; ^eJ_{4, 3}

Table 1

Table 2. ¹H-NMR chemical shifts of the oligosaccharides separated from sei whale milk

Reporter group	Residue	Chemical shifts, δ (coupling constants, Hz)			
		SW-1-1	SW-1-2	SW-1-3	SW-2
H-1	Glc α	5.220 (3.8)	5.224 (3.8)	5.221 (4.0)	5.222 (3.5)
	Glc β	4.660 (8.0)	4.668 (8.2)	4.665 (8.3)	4.665 (7.9)
	Gal ['] (β 1-4)	4.526 (8.0)	4.426 (7.6)	4.442	4.448 (7.9)
	Gal ^{''} (β 1-4)	-	-	4.456 (7.3)	-
	GlcNAc(β 1-3)	-	-	4.730 (6.8)	-
H-3ax	Neu5Ac(α 2-3)	1.794 (12.2 ^a , -12.2 ^b)	-	-	-
	Neu5Ac(α 2-6)	-	1.741 (12.2 ^a , -11.8 ^b)	1.722 (12.2 ^a , -12.7 ^b)	-
H-3eq	Neu5Ac(α 2-3)	2.750 (4.4 ^c)	-	-	-
	Neu5Ac(α 2-6)	-	2.709 (4.7 ^c)	2.670 (4.2 ^c)	-
H-3	Gal ['] (β 1-4)	4.111 (2.1 ^d)	-	-	-
H-4	Gal ['] (β 1-4)	-	-	4.158 (2.5 ^e)	-
NAc	GlcNAc(β 1-3)	-	-	2.053	-
	Neu5Ac(α 2-3)	2.027	-	-	-
	Neu5Ac(α 2-6)	-	2.026	2.028	-

^aJ_{ax, 4}; ^bJ_{3ax, 3eq}; ^cJ_{3eq, 4}; ^dJ_{3, 4}; ^eJ_{4, 3}

Table 2

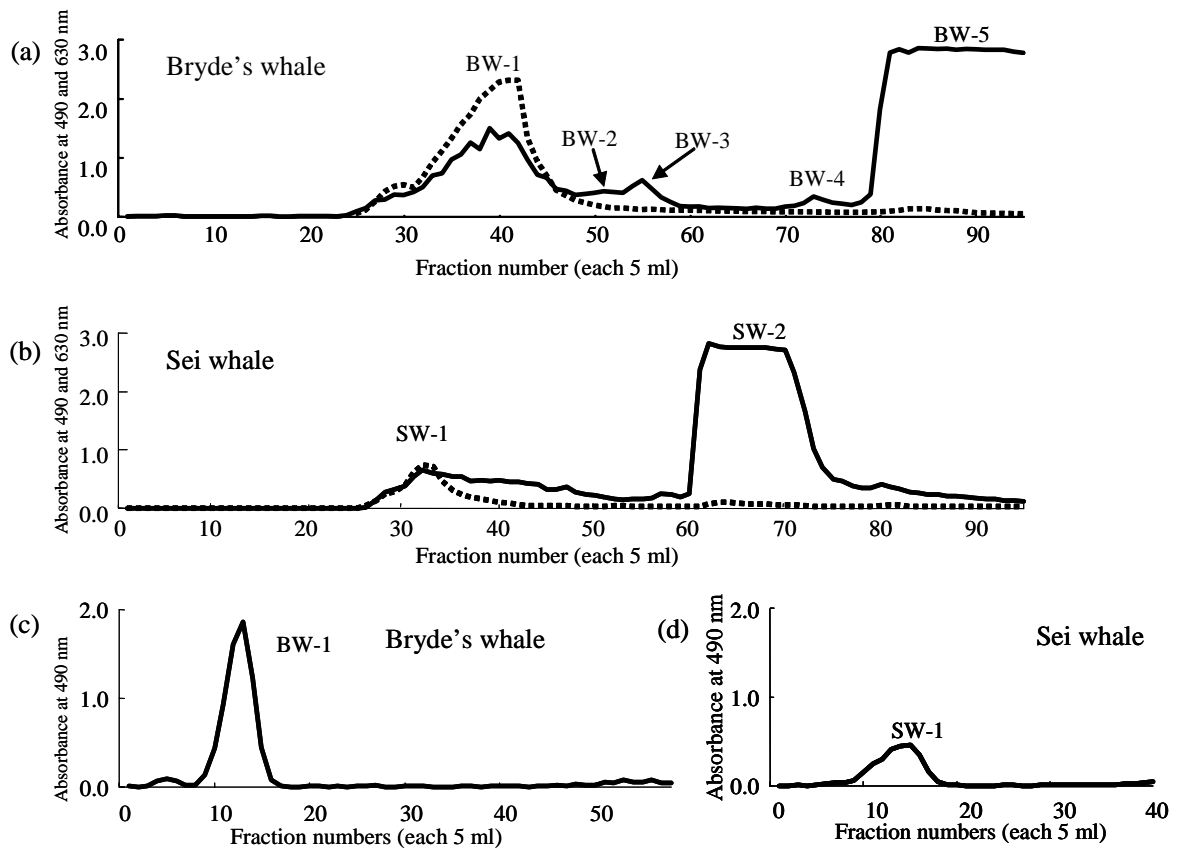


Fig. 1

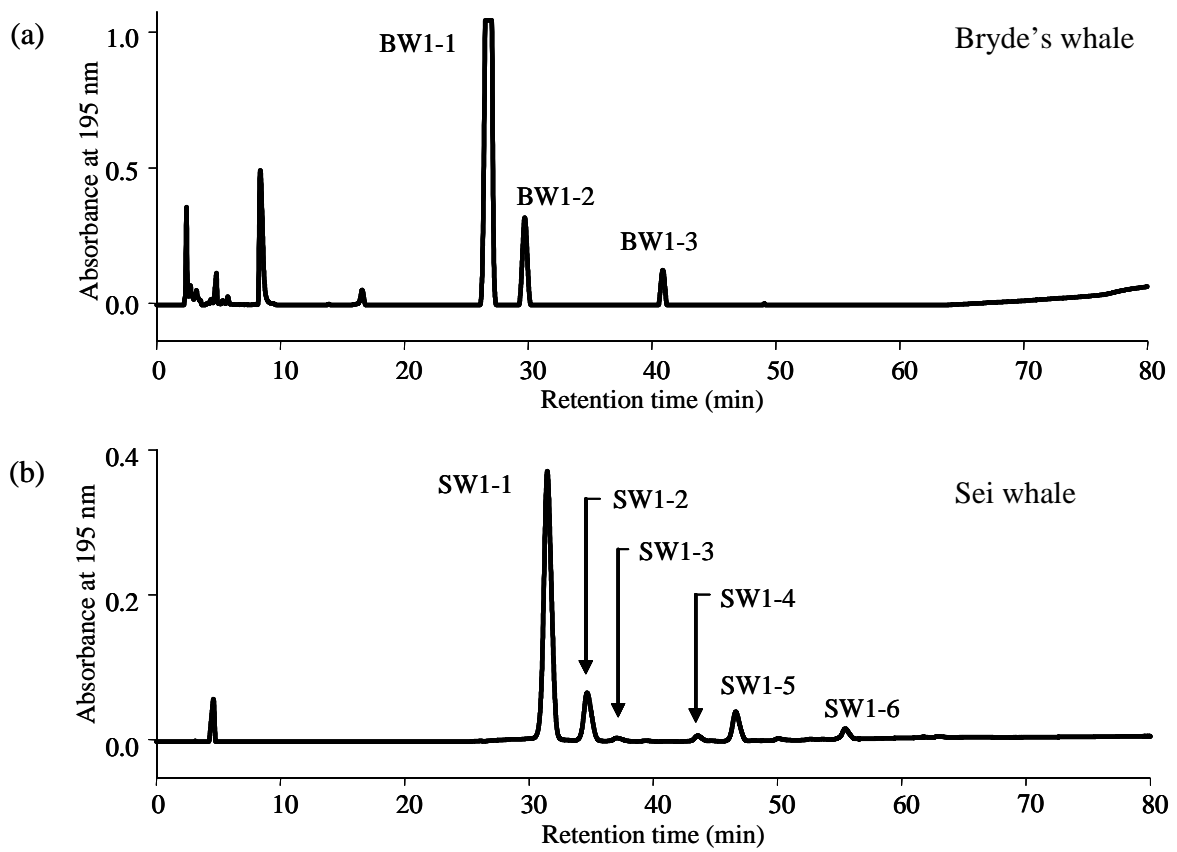


Fig. 2

	Bryde's wale	sei whale	minke whale	bottlenose dolphin
<i>Neutral oligosaccharides</i>				
				Gal(β1-4)Glc
				Gal(α1-4)Gal(β1-4)Glc
				Fuc(α1-2)Gal(β1-4)Glc
				GalNAc(α1-3)[Fuc(α1-2)]Gal(β1-4)Glc
				Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)Glc
				Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)Glc
<i>Sialyl oligosaccharides</i>				
				Neu5Ac(α2-3)Gal(β1-4)Glc
				Neu5Ac(α2-6)Gal(β1-4)Glc
				Neu5Ac(α2-3)[GalNAc(β1-4)]Gal(β1-4)Glc
				Neu5Ac(α2-3)Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)Glc
				Neu5Ac(α2-6)Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)Glc
				Neu5Ac(α2-3)Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)Glc

Fig. 3