Fisheries and Oceans Canada | Pêches et Océans Canada
Science | Sciences

Canadian Science Advisory Secretariat (CSAS)

Research Document 2013/120

Quebec Region

Temporal trends of PBDEs in adult and newborn beluga (*Delphinapterus leucas*) from the St. Lawrence Estuary

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April 2014

Canada

Foreword

This series documents the scientific basis for the evaluation of aquatic resources and ecosystems in Canada. As such, it addresses the issues of the day in the time frames required and the documents it contains are not intended as definitive statements on the subjects addressed but rather as progress reports on ongoing investigations.

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Published by:

Fisheries and Oceans Canada
Canadian Science Advisory Secretariat
200 Kent Street
Ottawa ON K1A 0E6

http://www.dfo-mpo.gc.ca/csas-sccs/
csas-sccs@dfo-mpo.gc.ca

ISSN 1919-5044

Correct citation for this publication:

Lebeuf, M., Raach, M., Measures, L., Ménard, N., and Hammill, M. 2014. Temporal trends of PBDEs in adult and newborn beluga (*Delphinapterus leucas*) from the St. Lawrence Estuary. DFO Can. Sci. Advis. Sec. Res. Doc. 2013/120. v + 11 p.

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ABSTRACT

In the 2008-2012 time period an unusually high number of stranded newborn beluga was recorded in the St. Lawrence Estuary (SLE). Blubber concentrations of polybrominated diphenyl ethers (PBDEs) in adult female beluga were not significantly different in 2008-2012 compared to the previous 10 years. Similarly, PBDE concentrations in stranded newborns in 2008-2012 were not significantly different than in previous time periods, 1995-2001 and 2002-2007, indicating no temporal trend. During the period 1995-2012, highest PBDE concentrations ever measured in adult females and in newborns were observed. Similar concentrations of PBDEs in adult females and newborns indicated that the transfer of PBDEs from mother to newborn was very efficient. PBDEs are known to have various endocrine disrupting effects on mammals including reproduction and development in offspring. However, direct and indirect association between the harmful effects of PBDE and the recent elevated frequency of complications at parturition and mortalities of newborns in stranded SLE beluga need further investigation.

Tendances temporelles des PBDE chez les bélugas (*Delphinapterus leucas*) adultes et nouveau-nés de l'estuaire du Saint-Laurent

RÉSUMÉ

Un nombre inhabituellement élevé de bélugas nouveau-nés échoués sur les rives de l'estuaire du Saint-Laurent (ESL) a été rapporté entre 2008 et 2012. Les concentrations de diphényléther polybromés (PBDE) mesurées dans le panicule adipeux de bélugas femelles adultes en 2008-2012 n'étaient pas significativement différentes de celles mesurées au cours des 10 années précédentes. Pareillement, les concentrations de PBDE chez les nouveau-nés échoués en 2008-2012 n'étaient pas significativement différentes des celles mesurées en1995-2001 et 2002-2007, indiquant l'absence de tendance temporelle. Entre 1995-2012, les concentrations de PBDE chez les bélugas femelles adultes et les nouveau-nés de l'ESL étaient à leur niveau maximum rapporté à ce jour. Des concentrations de PBDE similaires chez les femelles adultes et les nouveau-nés indiquent que les PBDE sont efficacement transférés entre la mère et son petit. Les PBDE sont des perturbateurs endocriniens reconnus affectant la reproduction et le développement de la progéniture chez les mammifères. Cependant, des études supplémentaires sont nécessaires afin de démontrer une association directe ou indirecte entre les effets nuisibles des PBDE et les taux élevés de complications à l'accouchement et de mortalités de nouveau-nés observés récemment chez les bélugas échoués de l'ESL.

INTRODUCTION

The population of beluga (*Delphinapterus leucas*) from the St. Lawrence Estuary (SLE) is considered threatened according to the Committee on the Status of Endangered Wildlife in Canada (COSEWIC 2004). Its decline resulted from exploitation during a large part of the 20th century, until 1979 when hunting ceased. Abundance indices of the population suggest that it is not recovering (Gosselin et al. 2014). Since the early 1980s, beluga mortalities documented in the SLE numbered, on average, 15 each year, the majority of which were adults (Measures 2008). Newborns were not found every year and averaged about one newborn yearly during the 1987-2007 time period. The situation drastically changed between 2008 and 2012, when more stranded newborns were found during this 5 year time period than in the previous 21 years (Lesage et al. 2014).

The Species at Risk Recovery Strategy for the SLE beluga indicated that chemical pollution is likely the most important threat to the population (DFO 2012). Concentrations of legacy persistent organic pollutants (POPs), such as PCBs and DDTs, in beluga decreased or remained constant since at least 1987 (Lebeuf et al. 2007). Polybrominated diphenyl ethers (PBDEs), however, have a different history as regulation is relatively recent, occurring in the late 1990s early 2000s in North America (Ward et al. 2008). A drastic rise in PBDEs was reported in beluga between 1987 and 1999 (Lebeuf et al. 2004). Preliminary results indicated that temporal trends of PBDE concentrations in adult SLE beluga changed in the late 1990's (Lebeuf et al. 2009). Contamination of newborn SLE beluga with PBDEs has not been studied.

A number of toxicological studies have demonstrated that exposure to PBDEs may have critical endocrine disrupting effects during fetal development (Letcher et al. 2010). The disruption of endocrine pathways by prenatal exposure to PBDEs may affect neurological development in exposed animals.

The main objective of this study is to provide an update on concentrations of PBDEs in blubber from adult male, adult female and newborn SLE beluga temporally. Four time periods were considered, namely, 1987-1994, 1995-2001, 2002-2007 and 2008-2012. This approach permitted analyses of the specific time period (2008-2012) when high mortalities of SLE newborn beluga were documented. The potential deleterious effects of PBDEs in recent mortality events of newborn beluga are discussed.

METHODS

SAMPLING

Samples were obtained from 78 adult male stranded beluga found on the shores of the SLE between 1988 and 2007, 76 adult females in the 1987-2012 time period and 27 newborns collected between 1995 and 2012. Standard length and age were used to define the two categories of beluga considered in this study. Adults were either 10 year old or > 3.0 m long whereas newborns were <1.7 m long. Standard length of each animal was measured from the rostrum to the notch of the tail fluke. Growth layer groups (GLGs), which are pairs of light and dark lines, were counted on longitudinal tooth sections but may have been underestimated due to difficulty in reading worn growth layers for some animals. According to Stewart et al. (2006), one GLG is deposited per year. In this study, age in years was reported for each adult beluga.

The state of preservation of carcasses was classified as very good, good, fair or poor (codes 1 to 4) (Geraci and Lounsbury 2005), although intermediate coding was also used (e.g. 2.5).

Details on blubber sampling were reported elsewhere (Lebeuf et al. 2007). In brief, a block of blubber extending from the skin to the muscle was collected, wrapped in solvent-rinsed aluminum foil and placed in a sealed plastic bag, and stored at -20°C until analysis. A subsample of the full depth of the blubber was taken from the block of blubber, homogenized and used for chemical analysis.

CHEMICAL ANALYSIS

Blubber samples were analyzed for PBDEs according to analytical methods used at the Maurice Lamontagne Institute, Department of Fisheries and Oceans Canada (Lebeuf et al. 2004, Raach et al. 2011). In brief, each blubber subsample was chemically dried with sodium sulfate before being transferred to a glass column. A single $^{13}C_{12}$ PCB (IUPAC number 170) was added to the column before the extraction procedure. Lipids and lipophilic compounds were extracted from the sample with dichloromethane (DCM). The extraction solution was split into three parts. The first part (c.a. 40% weight) was saved as a backup, the second part (c.a.10% weight) was used to gravimetrically determine the lipid content of the sample. The third part of the extraction solution received a mixture of up to four $^{13}C_{12}$ PBDEs as surrogate compounds, and was prepared for purification. Lipids were removed from the remaining extract by gel permeation chromatography using Bio-beads SX-3. The extract was further cleaned by elution through a two-layer column packed with neutral hydrated (5%) silica and alumina. The final extract was reduced in volume and spiked with an instrument performance solution containing two $^{13}C_{12}$ PCB (IUPAC numbers 111 and 189). Quantification of PBDEs was performed using a Varian 3400CX series gas chromatograph (Varian, Walnut Creek, CA, USA) equipped with a Varian Saturn IV ion trap, a Varian 1078 split/splitless programmable injector (5 µl injection volume) operated in splitless mode, and a Varian 8200CX autosampler or using a ThermoQuest Trace GC gas chromatograph (Thermo Fisher Scientific, Austin, TX, USA) equipped with a Finnigan PolarisQ ion trap, a ThermoQuest PTV split/splitless programmable injector (2 µl injection volume) operated in splitless mode, and a ThermoQuest AS2000 autosampler. The chromatographic separation of the PBDEs was achieved using a 30m DB-5MS column (0.25 mm ID, 0.25 µm film thickness (J&W Scientific, Folson, CA, USA)) with helium as the carrier gas. The ion source was operated in electron impact ionization mode and the ion trap in MS/MS mode. Concentrations of individual PBDE congeners were calculated using relative response factors determined from a five-point calibration curve. All reported concentrations were corrected for procedural losses of the surrogate compounds. PBDE concentrations refer to the sum of 11 PBDE congeners (IUPAC numbers 17, 28, 47, 49, 66, 99, 100, 153, 154, 155, 183).

QUALITY ASSURANCE AND QUALITY CONTROL

Samples were analyzed in batches of 10 samples, with one spiked blank and one certified reference material (SRM-1945), a blubber sample from a pilot whale (*Globicephala* sp.) obtained from the National Institute of Standards and Technology (NIST 2007). Reference material replicates (n=19) resulted in an average percent coefficient of variation of 11% for PBDEs, indicating a fairly high degree of reproducibility. The accuracy of analyses was -7% for the sum of 5 individual PBDE congeners (IUPAC numbers 47, 99, 100, 153, 154) for which certified values were provided for SRM-1945.

DATA ANALYSIS

A comparative approach based on four time periods, namely 1987-1994, 1995-2001, 2002-2007 and 2008-2012, was applied to biological and PBDE data from adult males, adult females and newborns. The time periods were selected to permit analyses of the specific time period (2008-2012) when high mortalities of newborns were documented in the SLE and to distribute as evenly as possible the number of beluga among time periods.

For each time period, biological variables were compared between adult males, adult females and newborns, when relevant, using Kruskal–Wallis (K-W) or Mann-Whitney (M-W) analysis for the carcass state of preservation and using analysis of variance (ANOVA) or a two-sample t test for age and standard length. Comparison of PBDE concentrations between adult males, adult females and newborns was assessed using either ANOVA or a two-sample t test for each time period.

Biological variables including age, standard length and carcass state of preservation were compared among time periods for adult males, adult females and newborns. ANOVA was used to compare age and standard length among time periods followed, when relevant, by a Bonferroni's post-hoc test. A K-W test was used to compare carcass state of preservation among time periods, when relevant the KW test was followed by ANOVA on ranked data and compared with Bonferroni's procedure. PBDE concentrations were expressed on a lipid weight basis and were natural logarithm (ln)-transformed to normalize the data distribution prior to statistical analysis. Comparison of PBDE concentrations among time periods for adult males, adult females or newborns were subjected to ANOVA followed by a Bonferroni's post-hoc test. Outliers were identified on the basis of studentized residuals exceeding the value of 3 and removed from the statistical analysis. All statistical analyses were performed using Systat 10 software (SPSS 2000) with statistical significance set at $\alpha = 0.05$.

RESULTS

BIOLOGICAL VARIABLES

Biological variables measured on beluga carcasses are reported in four time periods for adult females but only in three time periods for adult males and newborns as contaminant data are unavailable for the 2008-2012 and 1987-1994 time period, respectively (Table 1).

Statistical analysis showed that the mean age was significantly lower (t test; $p=0.04$) for adult males than adult females in the 1995-2001 time period. However, male carcasses were significantly longer than females within each time period. Adult male carcasses were on average in a poorer state of preservation than newborns (K-W; $p=0.007$) but only in the 1995-2001 time period.

Mean or ranked values for these variables were not significantly different among time periods in adult male, adult female or newborn beluga. Consequently, there was no significant temporal trend in age, standard length and state of preservation of carcasses in adult males over the 1987-2007 time period, in adult females over the 1987-2012 time period or in newborns over the 1995-2012 time period.

CONCENTRATIONS OF PBDEs

Mean concentration of PBDEs was significantly higher in adult males than adult females in the 1987-1994 time period. There was no significant difference of PBDE concentrations between adult males, adult females and newborns in the 1995-2001 and 2002-2007 time periods, but adult females were significantly (t test; p= 0.01) more contaminated than newborns in the 2008-2012 time period.

Mean concentrations of PBDEs in adult male, adult female and newborn beluga are reported for each time period in Figure 1. Adult males exhibited significant increasing concentrations of PBDEs temporally. However, adult females were on average significantly less contaminated only in the 1987-1994 time period compared to subsequent time periods. Concentrations of PBDEs in newborns were not significantly different among time periods.

DISCUSSION

BIOLOGICAL VARIABLES

In the 1995-2002 time period, adult males were, on average, younger than adult females by 7 years. In general, concentrations of POPs are believed to increase with age in beluga (Martineau et al. 1987). In this context, concentrations of PBDEs in adult males may be underestimated compared to adult females in that time period. However, considering the rapid increase of PBDEs in SLE beluga during the 1988-1999 time period (Lebeuf et al. 2004), the difference in age between adult males and females is likely negligible with respect to concentrations of PBDEs. Standard length was expected to differ between adult males and females. Males are longer than females in the SLE population as well as in other beluga populations. For instance, Muir et al. (1990) reported longer males than females in most beluga populations from Canadian waters and length data from SLE beluga collected in 1986-87 had similar mean and range values than beluga examined in this study. To the best of our knowledge, there is no demonstrated link between the standard length of a beluga and its contamination with POPs, assuming animals of similar ages, growing and feeding conditions. Differences in the average state of preservation of carcasses among categories of beluga were observed in the 1995-2001 time period (Table 1). Similar observations were reported by Lebeuf et al. (2007) but there is no obvious explanation for this. The significant differences in standard length and state of preservation of carcasses observed between males, females and newborns raise the question of effects of these variables on PBDE concentrations. There is at least one study supporting no effect of the state of preservation of carcasses on legacy POP concentrations in the blubber of cetaceans. Borrell et al. (2007) monitored concentrations of several legacy POP in blubber from harbour porpoises (*Phocoena phocoena*) over a 48 hour post-mortem period and found no significant change. Consequently, the effect of the state of preservation of carcasses with respect to PBDEs, is likely limited if not negligible.

Stranded beluga were selected on the basis of their age among samples available for PBDE analysis. As a result, no significant difference in age of adult males, adult females or newborn over time was observed among time periods. All the other biological variables examined in adult male and adult female beluga were not significantly different among time periods and consequently did not exhibit any temporal trend. Biological variables of newborns were not significantly different among time periods and did not exhibit any temporal trend in the 1995-2012 time period. The lack of temporal trend in age, standard length and state of preservation of carcasses of adult male, adult male, adult female or newborn carcasses support the fact that these biological variables did not influence the reported trends in PBDE concentrations.

PBDE CONCENTRATIONS AND TEMPORAL TRENDS

Beluga blubber is a reservoir for most lipophilic POPs. However, reproductive females transfer POPs to their offspring during lactation and gestation. As a result, blubber POP concentrations were generally lower in adult females than adult males (Martineau et al. 1987, Muir et al. 1990, Hoguet et al. 2013). However, PBDE concentrations were generally not significantly different between adults of both sexes, except for the 1987-1995 time period (Figure 1). Similar PBDE concentrations in blubber from adult SLE beluga have been previously reported and explained by the rapid increase of PBDEs in the SLE resulting from a much higher uptake of PBDEs by females than elimination during the 1988-1999 time period (Lebeuf et al. 2004).

Similar PBDE concentrations in adult females and newborns of SLE, although not based on newborn-mother pairs, suggested an efficient maternal transfer of PBDEs through gestation and lactation. Hoguet et al. (2013) confirmed highly efficient maternal transfer of PBDEs by reporting mean ratio of PBDE blubber concentrations (on a lipid basis) in a fetus to mother of 1.1 for three fetus-mother beluga pairs. Desforges et al. (2012) also showed similar PBDE blubber concentrations in fetus compared to mother for two fetus-mother beluga pairs despite the fact that the mass of PBDE transferred to fetus was relatively small (10%) compared to the mass burden in the mother.

Concentrations of PBDEs in blubber from adult female SLE beluga were much higher than adult female beluga from other populations. For instance, PBDE concentrations in adult female SLE beluga collected between 1995 and 2012 were in a higher range (n=52, mean=604 ng/g lip, range 222 - 1875), than an adult female (120 ng/g lip) or subadult beluga (n=6, mean=85.1 ng/g lip, range= 22.7-137) reported in beluga from Svalbard (Norway) sampled in 1999-2001 (Villanger et al. 2011). Concentrations of PBDEs in adult female beluga (n=2, mean= 5.5 ng/g lip) from Hendrickson Island (Canada) collected in 2008-2009 (Desforges et al. 2012) or female beluga (n=14, mean= 16 ng/g lip, range 4.4-59) from Eastern Hudson Bay (Canada) collected in 1999-2003 (Kelly et al. 2008) were also much lower than in SLE adult female beluga. Hoguet et al. (2013) analyzed PBDEs in blubber from females, mainly adults based on length, collected between 1989 and 2003 from two locations in Alaska (USA). PBDE concentrations in female beluga from Cook Inlet (n=12, range=7.4 – 32.0 ng/g lip) and Eastern Chukchi Sea (n=14, range= 1.9-19.4 ng/g lip) showed increasing temporal trends but maxima were still much lower than in adult female SLE beluga.

Significantly lower PBDE concentrations in adult SLE beluga were observed in the 1987-1994 time period compared to more recent time periods. A significant increase continued to be observed in males in most recent time periods, but the rate of increase was slower than in previous years. However, PBDE concentrations in adult female beluga in the most recent time period, 2008-2012, were not different than the two previous time periods. In females, no significant change of PBDE concentrations was observed since 1995-2001, consistent with an expected difference in PBDE concentrations between males and females (Figure 1). Concentrations of PBDEs in adult females and newborns remained constant during the 1995-2012 time period exhibiting maximum PBDE concentrations ever measured in these animals.

Predominant changes in PBDE temporal trends in adult SLE beluga were observed in late 1990's (Lebeuf et al. 2009). This is unexpected considering that the adoption of Canadian and

US legislative regulations for this group of chemicals occurred in the 2000's (Ward et al. 2008). Possible explanations are earlier regulations in some European countries (e.g. Germany) leading to a reduction of PBDEs through imported products, and/or the switch to alternative flame retardants by the industry prior to regulation such as a voluntary phase-out of PBDE production in North America (Ward et al. 2008).

POSSIBLE IMPLICATION OF PBDEs IN RECENT MORTALITY EVENTS OF NEWBORN SLE BELUGA

The exceptionally high number of mortalities of newborn SLE beluga in 2008-2012 raises a question on the role of toxic contaminants in these mortality events. As pointed out by Loseto and Ross (2011), it is particularly difficult to establish a cause-and-effect relationship between adverse health and exposure of marine mammals to toxic chemicals. A variety of approaches including associative, correlative, in vivo experimental and in vitro study designs have been applied. In general, no single approach provides sufficient evidence of causality for the effects of contaminants at the marine mammal population level. Most of the time, the 'weight of evidence approach' is suggested (Ross 2000).

Marine mammals, including beluga, are exposed to a variety of toxic chemicals and accumulated legacy POPs (Lebeuf 2009). PBDE concentrations in beluga reported in the 2008-2012 time period were still at their maximum concentrations in adult females and newborns and potentially at their maximum effect on health. The information on toxicity of PBDEs on marine mammals is, however, very limited and even more limited on beluga. To the best of our knowledge, Villanger et al. (2011) were the only workers to examine potential effects of PBDEs on beluga. They suggested disruptive effects of PBDEs on the thyroid function of beluga based on statistical relationships (correlative approach).

The toxicity of PBDEs has been reviewed by several authors (de Wit 2002; McDonald 2002; Darnerud 2003; Birnbaum and Staskal 2004). For mammals, including humans, effects of PBDEs are most often associated with thyroid hormone disruption which could result, among other effects, in reproductive problems and developmental neurotoxicity. Costa and Giordano (2007) reviewed the causal relationships between prenatal exposure to PBDEs and indices of developmental neurotoxicity observed in experimental animal models. They conclude that pre- or postnatal exposures of mice or rats to PBDEs cause long-lasting changes in spontaneous motor activity, mostly characterized as hyperactivity or decreased habituation, and disrupt performance in cognitive and memory tests. In their epidemiologic study, Herbstman et al. (2010) demonstrated inverse associations between elevated umbilical cord blood concentrations of PBDEs and adverse neurological developmental test scores in children.

The elevated mortalities of newborn beluga documented in the SLE in the 2008-2012 time period indicated that the cause was not due to infertility or early term death in utero. Newborn mortalities were post-partum, usually occurring within a few days or weeks after birth (Lair et al. 2014) suggesting a problem with the calf or the dam. If neurological damage affects the behavior and cognitive abilities of newborns, survival may be jeopardized. For example, difficulties may result from the newborn's failure to nurse or follow its mother, leading to abandonment and death. However, the hypothesis that newborns are deadly affected by PBDE exposure, as reflected by accumulated concentrations, does not coincide with the timing of these observations. In fact, concentrations of PBDEs in newborns have remained constant during the 1995-2012 time period whereas elevated mortalities occurred only since 2008-2012.

On the other hand, results from the necropsy program on the SLE beluga indicated that the occurrence of dystocias (difficult birth) or/ post-partum complications increased in the last decade of the study (2003-2012) compared to the two first decades (1983-2002) (Lair et al. 2014). This recent elevated occurrence of obstetric complications in SLE beluga coincides in time with maximum concentrations of PBDEs ever observed in adult female and newborn SLE beluga. Endocrine disrupting chemicals, such as PBDEs, have to be considered as a potential contributor to the elevated frequency of parturition complications observed recently in SLE beluga.

Risk factors for complications at parturition and neonatal mortalities of SLE beluga are numerous including other endocrine disrupting chemicals such as many legacy POPs and potential toxic chemicals present in the SLE but not yet known or reported. The effect of toxic chemicals may be indirect by promoting the effect of other stressors such as toxic algae (Tiedeken and Ramsdell 2009) or parasitism (Measures et al. 1995; Measures 2008; Marcogliese and Pietrock 2011). Further research, including monitoring of contaminants and their toxic effects, is critical in identifying important stressors that affect the SLE beluga population.

ACKNOWLEDGEMENTS

Thanks to Steve Trottier, Michelle Noël, Sylvie St-Pierre and Jenny D'Amours for their contribution to the analysis of PBDEs.

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Table 1. Characteristics of adult male, adult female and newborn beluga carcasses stranded on the shores of the SLE and analyzed for PBDEs. Mean ± std dev or median value, number of individuals in the calculation (in parentheses) and range [in brackets] are reported for each time period and compared[1] in each time period.

	1987-1994	1995-2001	2002-2007	2008-2012
Adult males	21	28	29	-
Age (year)	45±13 (20)	40^{a}±12 (28)	44±13 (27)	-
	[18-61]	[12-65]	[20-72]	
Standard length (m)	4.13^{a}±0.17 (21)	4.08^{a}±0.26 (28)	4.14^{a}±0.28 (29)	-
	[3.84-4.56]	[3.36-4.60]	[3.00-4.57]	
State of preservation of	3.5 (21)	3.5^{a} (28)	3.5 (29)	-
carcasses[2]	[2.0-4.0]	[2.5-4.0]	[2.0-4.0]	
Adult females	23	21	22	10
Age (year)	40±13 (23)	47^{b}±10 (20)	43±13 (22)	40±11 (10)
	[14-63]	[30-62]	[12-61]	[26-56]
Standard length (m)	3.58^{b}±0.16 (23)	3.66^{b}±0.20 (21)	3.61^{b}±0.20 (22)	3.62^{a}±0.22 (10)
	[3.29-3.92]	[3.31-4.01]	[3.10-3.91]	[3.06-3.91]
State of preservation of	2.5 (23)	3.0^{ab} (21)	3.3 (22)	2.5 (9)
carcasses	[2.0-4.0]	[2.0-4.0]	[2.0-4.0]	[2.0-3.5]
Newborns	-	6	6	15
Standard length (m)	-	1.57^{c}±0.12 (6)	1.55^{c}±0.09 (6)	1.56^{b}±0.09 (15)
		[1.39-1.68]	[1.44-1.70]	[1.369-1.68]
State of preservation of	-	2.0^{b} (4)	2.0 (6)	4.0 (10)
carcasses		[2.0-3.0]	[1.0-4.0]	[1.0-4.0]

[1] Refer to data analysis section for details on statistical tests, different letters indicate significant differences among adult male, adult female and newborn beluga in each time period. [2]According to the Geraci and Lounsbury (2005) classification.

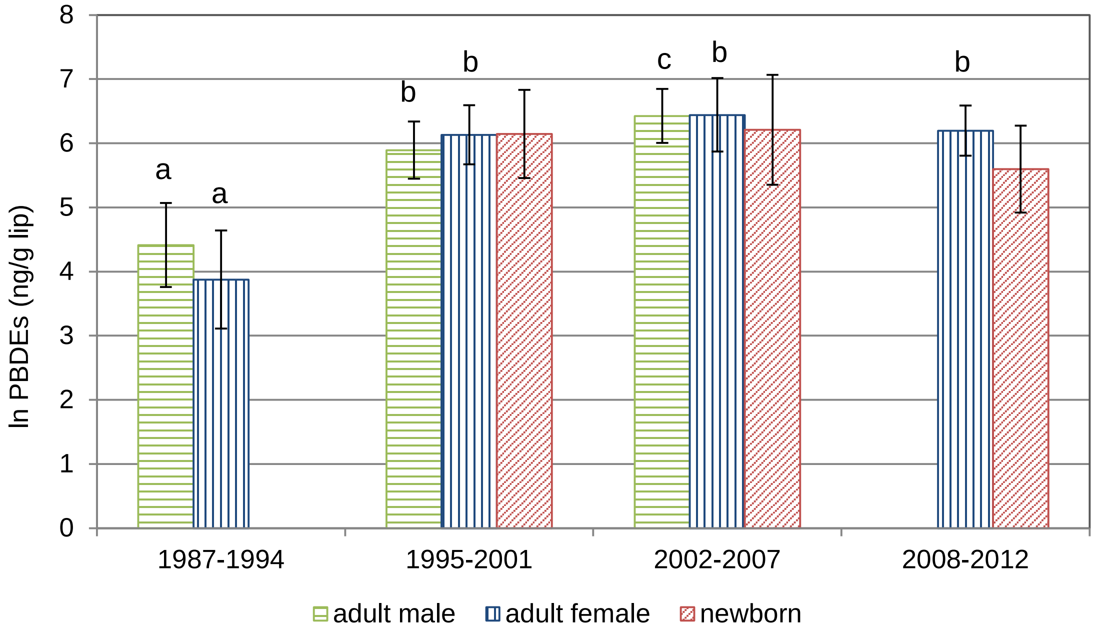

Figure 1. PBDE concentrations (ln ng/g lipid) (mean ± std dev) in the blubber from adult male, adult female and newborn beluga carcasses collected on the shores of the SLE for each indicated time period. Different letters for adult male and adult female beluga indicate significant differences among time periods. There was no significant difference among time periods for newborn beluga. Refer to data analysis section for details on statistical tests.