Journal Pre-proofs

Full length article

Variations in thyroid hormone levels in endangered st. Lawrence Estuary belugas: Potential linkage with stress and organohalogen contaminant exposure

Valérie Jolicoeur, Magali Houde, Lisa Loseto, Robert Michaud, Jonathan Verreault

PII:	S0160-4120(24)00233-2
DOI:	https://doi.org/10.1016/j.envint.2024.108647
Reference:	EI 108647
To appear in:	*Environment International*
Received Date:	20 December 2023
Revised Date:	15 March 2024
Accepted Date:	9 April 2024

Please cite this article as: V. Jolicoeur, M. Houde, L. Loseto, R. Michaud, J. Verreault, Variations in thyroid hormone levels in endangered st. Lawrence Estuary belugas: Potential linkage with stress and organohalogen contaminant exposure, *Environment International* (2024), doi: https://doi.org/10.1016/j.envint.2024.108647

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

© 2024 Published by Elsevier Ltd.

Variations in thyroid hormone levels in endangered St. Lawrence Estuary belugas: Potential linkage with stress and organohalogen contaminant exposure

Valérie Jolicoeur[a], Magali Houde[b], Lisa Loseto[c], Robert Michaud[d], Jonathan Verreault[a]*

[a] Centre de recherche en toxicologie de l'environnement (TOXEN), Département des sciences biologiques, Université du Québec à Montréal, P.O. Box 8888, Succursale Centre-ville, Montreal, QC, H3C 3P8, Canada

[b] Environment and Climate Change Canada, 105 McGill Street, Montreal, QC, H2Y 2E7, Canada

[c] Freshwater Institute, Fisheries and Oceans Canada, 501 University Crescent, Winnipeg, MB, R3T 2N6, Canada

[d] Group for Research and Education on Marine Mammals, Tadoussac, QC, G0T 2A0, Canada

*Corresponding author. E-mail address: verreault.jonathan@uqam.ca

Journal Pre-proofs

Abstract

The St. Lawrence Estuary (SLE) beluga (*Delphinapterus leucas*) population is highly exposed to an array of contaminants that were identified as one of the causes to the non-recovery of this endangered and declining population. In the last decade, an increasing number of parturition-associated complications and calf mortality has been reported in this population. It was suggested that elevated exposure to organohalogens (e.g., the halogenated flame retardants polybrominated diphenyl ethers [PBDEs]) and stress could play a role in this phenomenon by perturbing thyroid hormones. The objective of this study was to investigate the impact of concentrations of organohalogen contaminants and stress (cortisol levels) on thyroid hormone variations in adult male and female SLE belugas. Because plasma could not be collected in SLE belugas for ethical reasons, skin biopsy (n = 40) was used as a less-invasive alternative matrix to determine organohalogens (PBDEs and other halogenated flame retardants, polychlorinated biphenyls, and

organochlorine pesticides), cortisol, and thyroid hormones (triiodothyronine [T_3] and thyroxine [T_4]), and their metabolites reverse T_3 and 3,5-diiodothyronine [3,5-T_2]). Cortisol and thyroid hormones were analyzed by ultra-performance liquid chromatography-multiple reactions monitoring mass spectrometry (UPLC-MRM/MS). This method was compared using skin and plasma samples obtained from Arctic belugas. Comparisons of linear models showed that cortisol was a weak predictor for T_4, rT_3 and 3,5-T_2. Specifically, there was a weak significant negative association between T_4 and cortisol levels. Moreover, in male SLE belugas, a weak significant positive association was found between T_3 and Σ_{34}PBDE concentrations in skin. Our findings suggest that stress (i.e., elevated skin cortisol levels) along with organohalogen exposure (mainly PBDEs) may be associated with thyroid hormone level perturbations in skin of cetaceans.

Keywords: cetacean; thyroid hormone; cortisol; organohalogen contaminant; endocrine disruption.

1. Introduction

The St. Lawrence Estuary (SLE) beluga (*Delphinapterus leucas*) population (QC, Canada) inhabits a habitat highly impacted by human activities. This reproductively and geographically isolated population was drastically depleted by an intensive commercial hunt up until the mid-1950's and has not recovered despite the hunting ban in 1979 and implementation of several protective measures (Fisheries and Oceans Canada, 2024). This endangered population is facing significant anthropogenic threats including noise pollution, maritime traffic, reduction of prey availability and quality, habitat loss, and contaminant exposure (Fisheries and Oceans Canada, 2024). Moreover, increased rates of parturition-associated complications (e.g., dystocia) and mortality of calves since 2010 might accelerate the slow decline (~1% per year) observed in this population (Lair et al., 2016; Mosnier et al., 2015). Exposure to endocrine disrupting chemicals along with stress were specifically suggested by Lair et al. (2016) as potential contributors to these parturition-associated complications and perinatal mortality.

SLE belugas are chronically exposed to a wide variety of contaminants originating from the upstream populated and industrialized regions of the St. Lawrence River and Great Lakes (Canada and USA) where multiple pollutant sources can be found (e.g., industrial and municipal wastewater, and agricultural runoffs). Notably, SLE belugas were reported to accumulate in their blubber elevated concentrations of organohalogenated contaminants including organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs), and polybrominated diphenyl ethers (PBDEs) (Lebeuf et al., 2014; Simond et al., 2017, 2019, 2020). PCB and OCP concentrations in SLE beluga blubber remain worryingly elevated despite their ban in North America in the late 1970s (Lebeuf et al., 2014; Simond et al., 2017). As for PBDEs, while all commercial mixtures have been banned since 2009 (PentaBDE and OctaBDE) and 2017 (Deca-BDE), PBDEs represent the most abundant halogenated flame retardants (HFRs) measured in SLE belugas (Simond et al., 2017, 2019, 2020). Replacement products for the banned PBDEs (e.g., dechloranes,

hexabromobenzene [HBB], and pentabromoethylbenzene [PBEB]), short-chain chlorinated paraffins, per- and polyfluoroalkyl substances, as well as several emerging compounds including industrial antioxidants and UV absorbents have also been reported in SLE beluga tissues (Barrett et al., 2021; Blouin et al., 2022; Simond et al., 2020). Elevated tissue concentrations of organohalogens in SLE belugas are of health concern for this population as several of these chemicals have been linked to reproductive failure and endocrine disruption in marine mammals (reviewed by Sonne et al., 2020). As such, several organohalogens including PBDEs share structural similarities with thyroid hormones and were reported to disrupt the homoeostasis of these hormones through several mechanisms perturbing their synthesis, transport, action and metabolism in mammals (reviewed by Jugan et al., 2010).

In mammals, the thyroid prohormone thyroxine (T_4) and to a lesser amount the biologically active triiodothyronine (T_3) are produced by the thyroid gland. Most of T_3 production takes place in peripheral tissues through the deiodination of T_4 by iodothyronine deiodinases. Deiodination of thyroid hormones also produces metabolites such as the inactive reverse T_3 (rT_3) and 3,3-diiodothyronine (3,3'-T_2) as well as the weakly bioactive 3,5-diiodothyronine (3,5-T_2). Thyroid hormones play key roles in a range of physiological and developmental processes such as regulation of energetic metabolism, thermoregulation, growth, sexual development, and neurodevelopment (McNabb, 1992; Zoeller et al., 2007). Therefore, altered thyroid hormone homoeostasis can elicit adverse health effects including reproductive failure. For example, captive bottlenose dolphins (*Tursiops truncatus*) with failed pregnancy were reported to have significantly lower T_4 levels in serum at all stages of pregnancy compared to those that successfully gave birth (West et al., 2014). Contaminant exposure has been associated with decreased circulating thyroid hormone levels in several marine mammals including Norwegian Arctic (Svalbard) belugas (Villanger et al., 2011). Glucocorticoids, a class of corticosteroid hormones including cortisol that is predominant in cetaceans, were also shown to interact (i.e., crosstalk) with the thyroid axis in vertebrates (reviewed by Thambirajah et al., 2022). Glucocorticoids mediate physiological responses to stress including cardiovascular functions, immune system and inflammation, in addition to diverse metabolic and homeostatic changes (Pelt, 2011).

Nothing is known about thyroid hormone concentrations in tissues of the endangered SLE beluga population and their potential relationships with contaminant exposure and stress (cortisol levels). However, assessing hormone levels in large free-ranging marine mammals via capture and release methods can be challenging for technical and ethical reasons (Hunt et al., 2013), especially for species at risk like the SLE belugas. Recently, the analysis of several lipophilic hormones in skin and/or blubber biopsies based on liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) has been successfully validated for cetaceans including bottlenose dolphins and humpback whales (*Megaptera novaeangliae*), but limited to steroid hormones (e.g., cortisol, progesterone, and testosterone) (Boggs et al., 2017; Cates et al., 2019; Galligan et al., 2019, 2020). Skin biopsy may therefore represent a promising alternative matrix for the study of thyroid and glucocorticoid hormones and endocrine-disrupting chemicals including organohalogens in SLE belugas.

The objectives of this study were to: 1) develop a novel method for analyzing thyroid hormones (T_3, T_4, and their metabolites rT_3, 3,5-T_2 and 3,3-T_2) and cortisol in beluga skin using ultra-performance liquid chromatography-multiple reactions monitoring mass spectrometry (UPLC-MRM/MS) and enable comparisons between skin and plasma using samples from Arctic belugas, 2) measure the concentrations of selected organohalogens (PBDEs and other HFRs, OCPs, and PCBs), cortisol, and thyroid hormones in skin biopsies from SLE belugas, and 3) investigate relationships between these variables. We hypothesized that thyroid hormone levels are negatively associated with concentrations of selected organohalogens in SLE beluga skin and stress (i.e., higher skin cortisol levels). Results from this study will contribute to the development of alternative matrices for hormone determination in free-ranging marine mammals and deepen our understanding of the toxicity of organohalogens on the thyroid axis and potential interactions with stress.

2. Materials and Methods

2.1. Sampling

In September and October 2019, skin samples (epidermis and dermis) were collected from the flank of adult male (n = 20) and female (n = 20) belugas in the SLE (Fig. S1). Skin samples were obtained using a biopsy dart projector (model MK24c, Paxarms, Domett, Canterbury, New Zealand) loaded with 0.22 caliber blank charges and sharpened stainless-steel tips (8 × 25 or 8 × 35 mm) pre-cleaned with acetone, 95% ethanol, and Virkon (Lanxess, Cologne, North Rhine-Westphalia, Germany). Skin samples were sliced into two aliquots and placed in 2 mL cryogenic tubes immediately after collection using disposable DNAse and RNAse-free scalpels and cleaned forceps. One of the skin sample aliquots was immersed in dimethyl sulfoxide (DMSO), and both aliquots were kept on ice in a cooler in the field. The DMSO-stored sample was kept at 4°C until sexing. The sex of belugas was determined by polymerase chain reaction at Saint Mary's University (Halifax, NS, Canada) following methods based on Gilson et al. (1998). The other aliquot was transferred to a -30°C freezer in the laboratory until chemical and hormone analyses (sections 2.2\2.3 and 2.4, respectively). Sampling methods with SLE belugas were approved by the Institutional Animal Care Committee of the Université du Québec à Montréal (Montreal, QC, Canada) (permit no. 970), which complies with guidelines issued by the Canadian Council on Animal Care (Ottawa, ON, Canada). Sampling was conducted under permits granted by Parks Canada (SAGMP-2019-32682) and Fisheries and Oceans Canada (QUE-LEP-003-2019).

Samples of plasma and skin were also obtained from five Arctic belugas collected as part of a harvest monitoring program to assess the relevance of using skin as alternative to plasma for hormone determination. Samples were collected from the Eastern Beaufort Sea beluga population as part of the Hendrickson Island health and harvest monitoring program near Tuktoyaktuk in the Inuvialuit Settlement Region (NT, Canada). The program is supported by subsistence hunters from Tuktoyaktuk who kindly allowed samples to be collected from their landed whales as part of a

Journal Pre-proofs

collaborative monitoring effort run by the Tuktoyaktuk Hunters and Trappers Committee, the Fisheries Joint Management Committee, and Fisheries and Oceans Canada. For this study, the first five male belugas sampled from the 2018 Hendrickson Island program were used. These males were all mature and ranged in size from 410 to 450 cm. These tissue samples are referred to as Arctic beluga samples throughout the manuscript and were used to compare hormone levels between skin and plasma.

2.2. Thyroid hormone analysis

Thyroid hormones (T_3, T_4, rT_3, 3,5-T_2, and 3,3'-T_2) were analyzed in SLE beluga skin and Arctic beluga skin and plasma samples at the University of Victoria Genome BC Proteomics Centre (Victoria, BC, Canada).

2.2.1. Internal standard solution

An internal standard solution for skin samples was prepared by adding $^{13}C_6$-T_3 hydrochloride (Sigma-Aldrich, St. Louis, MO, USA) and $^{13}C_6$-T_4 (Sigma-Aldrich) to a 20% methanol-10 mM NaOH solution. This solution was diluted 10-fold with the same solution and was used as the internal standard solution for plasma samples and preparation of serially diluted, mixed standard solutions ($2{,}5\times10^{-6}$ to 1 µM each compound) of the five thyroid hormones (T_3 and T_4: Sigma-Aldrich; rT_3, 3,5-T_2, and 3,3'-T_2: Toronto Research Chemicals, Toronto, ON, Canada).

2.2.2. Sample preparation for skin samples

Minced skin aliquots (10-100 mg) were transferred to 2 mL safe-lock Eppendorf tubes. A skin pooled sample was also prepared similarly and used for quality control along the entire analytical procedure. An anti-oxidation buffer containing dithiothreitol (5 mg/mL) and ascorbic acid (5 mg/mL in water) and the internal standard solution for skin samples were added to each aliquot (1.5 µL/mg of tissue). The samples were homogenized using a MM 400 mixer mill (Retsch, Hann, North Rhine-Westphalia, Germany) and two 4 mm metal balls at a shaking frequency of 30 Hz for 1 min and cooled down for 1 min on ice between each homogenization. This procedure was repeated five times. Methanol was added to the samples (8 µL/mg of tissue), which were homogenized once again and sonicated in an ice-water ultrasonic bath for 5 min. The samples were then centrifuged (21,000 x *g*, 5°C, 10 min) and 100 µL of the clear supernatant of each sample was collected and mixed with 0.9 mL of NaOH (10 mM).

2.2.3. Sample preparation for plasma samples

Plasma aliquots (50 µL) and a pooled plasma sample used for quality control were mixed with 50 µL of an anti-oxidation buffer (see above) and 100 µL of the 10-fold diluted internal

standard solution. The plasma solution was mixed with a vortex and 800 μL of methanol was added to the solution. The mixture was sonicated in an ice-water ultrasonic bath for 5 min before centrifugation (21,000 x *g*, 10 min). The supernatant (300 μL) was collected and mixed with 2.7 mL of NaOH (10 mM).

2.2.4. Sample clean up

The skin and plasma samples were subsequently cleaned up by solid phase extraction using a polymeric reversed-phase solid-phase extraction Strata-X cartridge (200 mg/3 mL, Strata, Phenomenex, Torrance, CA, USA), which was activated with 2 mL of methanol and preconditioned with 10 mM NaOH. The samples were loaded on the cartridges and percolated through the cartridge under a positive pressure. The flow-through fraction was discarded, and the cartridge was washed with 3 mL of 10 mM NaOH. Analytes were eluted with 2 mL of 95% methanol-10 mM NaOH solution. The collected fraction was then dried in a speed-vacuum concentrator. The residues from the skin samples were dissolved in 50 μL of a 20% methanol-10 mM NaOH solution, while the residues from the plasma samples were diluted in 50 μL of a 20% methanol-10 mM NaOH solution. The resultant solutions were clarified by centrifugation at 21,000 x g at 5°C for 5 min.

2.2.5. UPLC-MRM/MS

Aliquots (10 μL) of the clear sample solutions were injected onto an Acquity UPLC system (Waters Corporation, Milford, MA, USA) coupled to a QTRAP 6500+ MS (AB Sciex, Framingham, MA, USA). The MS was operated in the positive ion detection mode. Separation of analytes was performed on a BEH C18 column (2.1 × 100 mm × 1.7 μm, Waters Corporation) at 45°C and flow rate of 0.3 mL/min. A mobile phase, which was composed of 0.2% formic acid in water (solvent A) and 0.2% formic acid in acetonitrile (solvent B), was used for binary-solvent gradient elution (20% to 80% B in 10 min). The quality control solutions that were prepared from the pooled samples were injected at the beginning, middle, and end of UPLC-MRM/MS batch runs to monitor the stability of analytes in solution and the LC-MS instrument throughout the batch runs. The acquired UPLC-MRM/MS data were processed using the Sciex MultiQuant software suite. Linear-regression internal standard calibration curves of thyroid hormones were constructed with the data acquired from injections of the serially diluted standard solutions. Concentrations of thyroid hormones detected in the samples were calculated by interpolating the calibration curves of individual thyroid hormones with the analyte-to-internal standard peak area ratios measured from injection of the sample solutions. Method limits of quantification (MLOQs) can be found in Table S1 (see Appendix S1 for methods). The mean coefficients of variation (standard deviation divided by the mean) of the quality control sample injections (n = 4 for skin; n = 3 for plasma) were as follow: 3,3'-T_2: 19%; 3,5-T_2: 3%; rT_3: 8%; T_3: 5%; and T_4: 5%.

2.3. Cortisol analysis

Cortisol and 10 other steroids (see full list in Table S2) were analyzed in beluga skin and plasma samples at the University of Victoria Genome BC Proteomics Centre.

2.3.1 Internal standard solution

An internal standard solution containing ^{13}C- or deuterium-labeled steroid hormones, including pregnenolone-d4, 17α-hydroxypregnenolone-d3, progesterone-d9 (CDN Isotopes Inc., Pointe-Claire, QC, Canada) and 11-deoxycorticosterone-d8, corticosterone-d4, cortisol-d4, aldosterone-d4 and androstenedione-$^{13}C3$ (Sigma-Aldrich) was prepared in 50% acetonitrile. Next, a mixed solution of standards of the targeted steroid hormones was prepared in the internal standard solution. This solution was serially diluted with the same internal standard solution to have 10-point calibration solution in a range of 0.001 to 1000 nM for each hormone.

2.3.2. Sample preparation for skin samples

Each skin sample was weighed into a 2-mL tissue homogenizing tube and 10 µL of PBS buffer containing 2% phosphoric acid per mg of raw tissue and two 5-mm metal beads were added to each tube. Each sample was homogenized on a MM 400 mixer mill (Retsch) at 30 Hz until the tissue aliquot was completely homogenized. An aliquot of the homogenates, equivalent to 20 mg of the raw tissue for each sample, was then transferred to another 2-mL homogenizing tube, and then 100 µL of the internal standard solution and 400 µL of acetonitrile was added. The mixture was homogenized again for 2 min, which was repeated three times, followed by centrifugal clarification at 21,000 × *g* at 10°C for 10 min. The clear supernatant of each sample was taken out and transferred to a 5-mL borosilicate glass test tube, and 800 µL of ethyl acetate and 400 µL of 200-mM ammonium acetate solution (pH 6) were added to each tube. The mixture was vortexed for 1 min at 3,000 rpm on a Fisherbrand digital vortexer and then centrifuged at 3,000 x *g* for 10 min in a Beckman Allegra R-22A centrifuge. The clear supernatant of each sample was collected. The liquid-liquid extraction was repeated twice and the pooled extractant of each sample was then dried under a nitrogen gas flow. The residues were reconstituted in 100 µL of 50% acetonitrile. After sonication for 1 min and centrifugation at 21,000 × *g* for 1 min, 10 µL of the clear supernatant was injected onto the UPLC-MRM/MS for quantitation.

2.3.3. Sample preparation for plasma samples

50 µL of plasma was added with 50 µL of the internal standard solution and 300 µL of 200-mM ammonium acetate buffer (pH 6). After vortex-mixing at 3,000 rpm for 10 sec on a Fisherbrand digital vortexer, 800 µL of ethyl acetate was added to each tube. The mixture was vortex-mixed for 1 min followed by centrifugal clarification at 21,000 × *g* at 5°C for 5 min. The clear supernatant of each sample was carefully taken out and transferred to a LC micro-injection vial. The content in the vial was dried under nitrogen and the residues were reconstituted in 50 µL of 50% acetonitrile. After sonication for 1 min and centrifugation at 21,000 × *g* for 1 min, 10 µL of the clear supernatant was injected onto the UPLC-MRM/MS for quantitation.

2.3.4. UPLC-MRM/MS

Aliquots (10 µL) of clear supernatant samples were injected onto an Acquity UPLC system (Waters Corporation) coupled to a QTRAP 6500+ MS (Sciex) operating in positive ion detection mode. Separation of analytes was performed on an Agilent Eclipse C18 column (2.1 × 150 mm × 1.8 µm; Agilent Technologies Inc.) and the mobile phase was 0.05% formic acid in water (solvent A) and 0.05% formic acid in acetonitrile (solvent B) for gradient elution: 25% to 75% B over 15 min, followed by 100% B for 2 min, and column equilibration at 25% B for 4 min between injections. The column flow was 0.3 mL/min, and the column temperature was 50°C.

The quality control pooled samples were injected at the beginning, middle, and end of UPLC-MRM/MS batch runs to calculate in-batch CV% to ensure repeatability of the quantitation. The linear-regression calibration curves of cortisol and the other steroids were constructed with the data acquired from injections of the serially diluted calibration solutions in an appropriate concentration range for each steroid. Concentrations of the steroids detected in samples were obtained by interpolating the linear calibration curves with the analyte-to-internal standard peak area ratios measured from injections of the sample solutions. The MLOQs can be found in Table S2 (see Appendix S1 for methods). The mean coefficient of variation (standard deviation divided by the mean) of the quality control sample injections (n = 4 for skin samples) for cortisol was 14.5% (Table S3; all other steroids analyzed are also listed therein).

2.4. Chemical analyses

SLE beluga skin samples were extracted, cleaned-up, and analyzed for 35 PBDEs and 12 other HFRs at Université du Québec à Montréal (Montreal, QC, Canada); 41 PCBs and 31 OCPs were analyzed at AGAT Laboratories (Montreal, QC, Canada) (Tables S4-S7). Methods for extraction, clean-up, and analysis of SLE beluga skin samples have been described by Simond et al. (2017) and were applied with minor modifications. Briefly, skin sample aliquots (40-50 mg) were homogenized with diatomaceous earth (J.T. Baker, Phillipsburg, NJ, USA) and spiked with 100 µL of a 200 ng/mL internal standard solution (BDE-30, BDE-156, ^{13}C-BDE-209, and ^{13}C-*anti*-DP) for HFRs, 4 µL of a 100 ng/mL performance standard solution (CB-16, -65, -166, and -200) for PCBs, and 20 µL of a 1 µg/mL performance standard solution (2,4,5,6-Tetrachloro-m-xylene) for OCPs. Sample extraction was carried out using a pressurized liquid extraction system (Fluid Management Systems, Billerica, MA, USA) using dichloromethane and *n*-hexanes (50:50, volume ratio). The extracted samples were further cleaned-up using PBDE-free acid-basic-neutral silica followed by neutral alumina columns (Fluid Management Systems). The lipid content of each sample was determined gravimetrically. PBDEs and other HFRs were identified and quantified using a gas chromatograph (GC) coupled to a single quadrupole MS (Agilent Technologies 5975C Series, Palo Alto, CA, USA) operating in electron capture negative ionization mode (ECNI). The analytical column was a fused silica DB-5 HT capillary column (15 m × 0.25 mm × 0.10 mm; J & W Scientific, Brockville, ON, Canada).

The final cleaned skin sample extracts were subsequently sent to AGAT laboratories for PCB and OCP analyses. PCBs and OCPs were identified and quantified using a 6890N GC coupled to a 5975B inert mass selective detector (MSD), while a 7890A GC coupled to a 5975C MSD (Agilent Technologies) was used for OCPs. Both GC/MSD were equipped with a fused silica RXI-5SIL MS capillary column (30 m × 0.25 mm × 0.25 µm) (Restek Corporation, Bellefonte, PA, USA) and were operated in selected ion monitoring (SIM) mode.

Quality control and assurance procedures included the analysis of procedural method blanks and standard reference material (NIST 1945 Whale Blubber, Gaithersburg, MD, USA) for each batch of ten samples. The mean percentage of variation from certified concentrations in standard reference material was 7% for PBDEs (4 congeners), 27% for PCBs (26 congeners) and 49% for OCPs (3 compounds). We excluded CB-209 and *p,p'*-DDE from further analyses because of high variations between measured concentrations and certified values in reference material. The recoveries (mean ± SD) of internal standards were as follows: BDE-30: 99 ± 6%, BDE-156: 95% ± 8, ^{13}C-BDE-209: 36 ± 9%, ^{13}C-*anti*-DP: 97 ± 9%. BDE-209 was excluded from further analyses due to low percent recovery in skin samples. The recoveries (mean ± SD) of performance standards for PCBs and OCPs were: CB-16: 86 ± 17%, CB-65: 96 ± 19%, CB-166: 96 ± 18%, CB-200: 99 ± 17%, 2,4,5,6-Tetrachloro-m-xylene: 81 ± 23%. Concentrations of PBDEs and other HFRs were inherently recovery-corrected since quantification was based on an internal standard method, while concentrations of PCBs and OCPs were not recovery-corrected. Analytes that were detected in blanks at concentrations exceeding 5% of the mean sample concentrations were blank-corrected in all samples (i.e., BDE-10, -28/Polybutylene terephthalate [PBT], -66, and -77, PBEB, and HBB). Analyte concentrations are reported in ng/g wet weight because no correlations were found between the lipid content of the samples and the analyte concentrations. The method limits of detection (MLODs) and\or MLOQs (see Appendix S1 for methods) are listed in Tables S4-S7.

2.5. Statistical analysis

Values below MLODs or MLOQs for organohalogens were assigned a concentration of zero and individual organohalogenated compounds or congeners were summed into their respective chemical classes (Σ_{40}PCB, Σ_{29}OCP, Σ_{34}PBDE, and other HFRs as Σ_{12}HFR). Non-detects for hormones were replaced with a value computed using *NDexpo* (Lavoué, 2019) before generating the mean. This web application uses a method based on the robust regression on order statistics approach to replace censored data with values estimated assuming a log-normal distribution. Only the organohalogen classes and individual hormones (cortisol, T_3, T_4, rT_3, 3,5-T_2, and 3,3'-T_2) that were detected in more than 65% of the samples were included in statistical analyses. The normality of the data was verified using the Shapiro-Wilk test (Table S8). Variables were transformed with the natural logarithm (ln) when needed to meet this assumption. Homogeneity of variance was tested using Bartlett's test (Table S8). Differences in variable means between SLE beluga males and females were tested using the Student's T tests when the assumptions were met, otherwise the non-parametric Wilcoxon signed-rank test was used.

To investigate the potential effects of organohalogens and stress (cortisol) on thyroid hormone levels, we built 16 multiple linear regression candidate models explaining thyroid hormone level variations in SLE beluga skin based on a priori hypotheses (Table S9). A null model (intercept-only) was included to assess the relative support in favor of models compared to the null model. The homoscedasticity and normality of residuals were verified with the most complex models. Hormone levels were either ln-transformed (T_3, rT_3, 3,5-T_2, T_3/T_4, and cortisol) or transformed using the square root (T_4) to meet these assumptions. Multicollinearity was assessed for each predictor using the variance inflation factor (VIF) score (Table S10). Variables with a VIF higher than 2.5 were not included in the same model. Candidate models were compared using the Akaike information criterion corrected for small sample sizes (AICc) using the *AICcmodavg* package (Mazerolle, 2020). Correlations between lipid contents, organohalogen contaminant concentrations and hormone levels were also investigated using the Spearman's rank test. All statistical analyses were performed in R 4.1.1 (R Core Team, 2021).

3. Results

3.1. Hormone concentrations in Arctic belugas

Thyroid hormones and their metabolic products (T_3, T_4, rT_3, and 3,5-T_2) as well as cortisol (and other steroids) were quantified in plasma and skin of the five Arctic beluga males, while 3,3'-T_2 was not detected in any samples (Table S11). No statistical analysis was performed due to low sample size. Expectedly, plasma showed the highest concentrations of T_4 and cortisol compared to the skin (Table S11). The T_4/T_3 level ratios were higher in plasma than in skin (~12-fold) (Table S12). Qualitatively, there were no apparent relationships between skin and plasma levels for T_3, T_4, T_4/T_3 level ratios, and cortisol (Fig. S2-S5).

3.2. Hormone concentrations in SLE belugas

The T_3, T_4 and their metabolites 3,5-T_2 and rT_3 were all successfully quantified in more than 65% of the SLE beluga skin samples (100, 100, 85, and 70% of individuals, respectively), while 3,3'-T_2 was only quantified in a few individuals (25%). The most abundant thyroid hormones quantified in skin were in combined sexes T_4 (10.2 ± 0.73 pmol/g ww; mean ± SEM) followed by T_3 (1.43 ± 0.20 pmol/g ww), while 3,5-T_2 (0.34 ± 0.05 pmol/g ww) and rT_3 (0.24 ± 0.04 pmol/g ww) were measured at much lower levels (30- and 43-fold lower than T_4, respectively) (Table 1; for males and females separately). The skin T_3 and T_4 levels strongly correlated with their respective deiodinated products, namely 3,5-T_2 (Spearman's $\rho = 0.92$, $p < 0.001$) and rT_3 (Spearman's $\rho = 0.72$, $p < 0.001$). There was also a moderate negative correlation between T_3 and rT_3 (Spearman's $\rho = -0.42$, $p = 0.007$). The mean concentration of cortisol in SLE beluga skin was 0.53 ± 0.07 pmol/g ww (Table 1; for males and females separately). There was no difference

between males and females for the concentrations of thyroid hormones and their metabolites or neither those of cortisol (Table S13). The T_4/T_3 level ratios were significantly lower (~2-fold) in SLE beluga females than males (9.97 ± 2.50 and 20.15 ± 6.39, respectively; t = -2.49, p = 0.02) (Table S13).

Table 1. Mean concentrations (± SEM and range; pmol/g ww) of thyroid hormones and their metabolites as well as cortisol in biopsied skin of male and female SLE belugas. Method limits of quantification (MLOQs) are listed in Table S1.

	Male SLE belugas	**Female SLE belugas**
	n = 20	n = 20
T_3	0.99 ± 0.15 (0.07 – 2.85)	1.86 ± 0.34 (0.29 – 6.10)
T_4	10.5 ± 0.71 (4.85 – 15.2)	9.83 ± 1.28 (1.09 – 28.7)
rT_3	0.29 ± 0.05 (<MLOQ – 0.76)	0.19 ± 0.04 (<MLOQ – 0.68)
3,5-T_2	0.22 ± 0.05 (<MLOQ – 0.64)	0.46 ± 0.09 (<MLOQ – 1.39)
Cortisol	0.38 ± 0.08 (0.03 – 1.25)	0.67 ± 0.09 (0.07 – 1.26)

3.3. Organohalogen concentrations in SLE belugas

The most abundant organohalogen classes measured in skin were Σ_{40}PCB (257 ± 28 ng/g ww; mean ± SEM) and Σ_{29}OCP (46 ± 4 ng/g ww), accounting for 81% and 14% of the sum of all organohalogens, respectively, while Σ_{34}PBDE (15 ± 1.36 ng/g ww) accounted for 5% (Table 2; for males and females separately). Other HFRs (i.e., HBB and PBEB) contributed to less than 1%

Journal Pre-proofs

of the sum and were only detected in 60% of the SLE beluga skin samples; as a result, they were not included in subsequent statistical analyses (Table S7). The most abundant congeners for PCBs and PBDEs were CB-153 (17% of Σ_{40}PCB) and BDE-47 (60% of Σ_{34}PBDE), respectively (Tables S4 and S6). Only two OCPs were quantified: HCB and trans-nonachlor (39% and 61% of the total detection, respectively; Table S5).

Table 2. Mean lipid content (%) and concentrations (± SEM and range; ng/g ww) of organohalogenated contaminants in biopsied skin of male and female SLE belugas.

	Male SLE belugas	**Female SLE belugas**
	n = 20	*n* = 20
Lipid content (%)	8.12 ± 0.91 (1.90 – 16.6)	8.48 ± 0.99 (2.94 – 16.6)
Σ_{40}PCB[a]	265 ± 38.8 (<MLOQ – 613)	250 ± 46.5 (<MLOQ – 681)
Σ_{29}OCP[b]	44.6 ± 5.2 (5 – 95)	46.7 ± 5.9 (8 – 95)
Σ_{34}PBDE[c]	15.9 ± 2.02 (2.40 – 33.7)	13.7 ± 1.83 (1.92 – 31.0)

[a] Sum of CB-17, -18, -28, -31, -44, -49, -52, -70, -74, -87, -95, -99, -101, -105, -110, -118, -128, -132, -138, -149, -151, -153, -158, -169, -170, -171, -177, -180, -183, -187, -191, -199, -206, and -208. Congeners that were not detected in any samples: CB-33, -82, -156, -195, -194, and -205. CB-209 was excluded from the analyses (see section 2.4).

[b] Sum of HCB and trans-nonachlor. Pesticides that were not detected in any samples: alachlor, aldrine, α- and *γ*-chlordane, oxychlordane, dimethyl tetrachloroterephthalate, dieldrin, α- and β-endosulfan, endosulfan sulfate, endrin, endrin aldehyde, endrin ketone, heptachlor, heptachlor epoxyde, α-, β -, γ- and δ -hexachlorocyclohexane, imidacloprid, methoxychlor, mirex, octachlorostyrene, *p,p*'-dichlorodiphenyldichloroethane, *p,p'*-dichlorodiphenyltrichloroethane, *trans*- and *cis*-permethrin. *p,p*'-DDE was excluded from the analyses (see section 2.4).

[c] Sum of BDE-7, -10, -17, -28/PBT, -47, -49, -66, -77, -85, -99, -100, -126, -154/2,2',4,4',5,5'-hexabromobiphenyl [BB-153], -153, and -203. Congeners that were not detected in any samples:

Journal Pre-proofs

BDE-15, -71, -119, -138, -139, -140, -171, -180, -183, -184, -191, -196, -197, -201 -204, -205, -206, -207, and -208. BDE-209 was excluded from the analyses (see section 2.4).

3.4. Associations between thyroid hormones, cortisol and organohalogens

The top-ranked linear models explaining the levels of T_3, T_4, rT_3, 3,5-T_2, and T_4/T_3 level ratios in SLE beluga skin generally obtained a moderate or weak support (AICc weight range: 14-32%; Table 3). The model-averaged estimates and 95% confidence intervals for predictors in all models that ranked within a ΔAICc of 2 from the top-ranked model are listed in Table S14. Cortisol was the only predictor in the first-ranked model explaining levels of T_4 (AICc weight: 37%). The linear model (adjusted $R^2 = 0.10$, $F_{1,38} = 5.22$, $p = 0.03$) showed a weak negative association between cortisol and T_4 concentrations in SLE beluga skin (Table 4 and Fig.1). Cortisol was also a weak significant predictor in the first-ranked models explaining levels of rT3 (adjusted $R^2 = 0.09$, $F_{1,38} = 4.91$, $p = 0.03$), 3,5-T_2 (adjusted $R^2 = 0.13$, $F_{1,37} = 3.96$, $p = 0.03$), and T_4/T_3 (adjusted $R^2 = 0.24$, $F_{1,37} = 4.91$, $p = 0.01$) (Table 4).

The predictors in the first and second-ranked models for levels of T_3 were sex and Σ_{34}PBDE concentrations. However, the intercept-only model was within an AICc score < 2 from the top ranked models for T_3 levels. This suggests that the top-ranked models did not substantially outperform the null model in explaining T_3 variations.

Table 3. Highest ranked linear models (ΔAICc < 2) explaining thyroid hormone levels in SLE beluga skin (n = 40).

T_3	K	AICc	ΔAICc	w
T_3 ~ Sex	3	106.82	0.00	0.15
T_3 ~ Σ_{34}PBDE + Sex	4	107.05	0.24	0.14
T_3 ~ Cortisol	3	107.63	0.81	0.10

T_3 ~ Cortisol + Sex	4	108.02	1.20	0.08
T_3 ~ Intercept only	2	108.10	1.28	0.08
T_3 ~ Σ_{29}OCP + Sex	4	108.74	1.92	0.06
T_4				
T_4 ~ Cortisol	3	86.59	0	0.37
rT_3				
rT_3 ~ Cortisol	3	124.44	0	0.29
rT_3 ~ Cortisol + Sex	4	125.92	1.48	0.14
3,5-T_2				
3,5-T_2 ~ Cortisol + Σ_{115}OHC	4	131.81	0.00	0.21
3,5-T_2 ~ Cortisol	3	132.27	0.46	0.16
3,5-T_2 ~ Cortisol + Σ_{115}OHC + Sex	5	133.11	1.30	0.11

Journal Pre-proofs

3,5-T_2 ~ Cortisol + Sex	4	133.31	1.49	0.10
3,5-T_2 ~ Sex	3	133.75	1.94	0.08

T_4/T_3 ratio

T_4/T_3 ~ Cortisol +Sex	4	106.20	0	0.29
T_4/T_3 ~ Cortisol	3	106.69	0.48	0.22
T_4/T_3 ~ Cortisol + Σ_{115}OHC + Sex	5	107.80	1.60	0.13
T_4/T_3 ~ Cortisol + Σ_{115}OHC	4	108.02	1.82	0.11

K: Number of estimated parameters.

AICc: Second order Akaike information criterion.

ΔAICc: Difference in AICc score between the current model and the top-ranked model.

w: Akaike weight.

Σ_{115}OHC: Sum of all PBDEs and other HFRs, PCBs, and OCPs.

Journal Pre-proofs

Table 4. Most parsimonious linear regression model predicting hormone levels in male and female SLE belugas (*n* = 40) and T_3 in males only (*n* = 20). Hormone levels were log-transformed.

Hormone	Predictor	Coefficient Estimate	Standard error	*t*	p

T_4	Intercept	2.86	0.15	18.68	< 0.01
	Cortisol	-0.23	0.10	-2.84	0.03
rT_3	Intercept	-2.36	0.25	-9.59	< 0.01
	Cortisol	-0.36	0.16	-2.22	0.03
3,5-T_2	Intercept	-1.65	0.35	-4.67	< 0.01
	Cortisol	0.47	0.18	2.60	0.01
	Σ_{115}OHC	0.00	0.00	1.68	0.10
T_4/T_3 ratio	Intercept	1.64	0.21	7.70	< 0.01
	Cortisol	-0.30	0.13	-2.22	0.03
	Sex (Male)	0.48	0.29	1.69	0.10
T_3 (males only)	Intercept	-0.94	0.34	-2.80	0.01
	Σ_{34}PBDE	0.04	0.02	2.33	0.03

We conducted other AICc comparisons of linear regression models predicting T_3, rT_3, 3,5-T_2 and T_4/T_3 levels for male and female belugas separately (Table S15-S16). The predictor in the most parsimonious model for T_3 in males was Σ_{34}PBDE (AICc weight: 0.49). This model suggests a weak significant positive association between T_3 and Σ_{34}PBDE concentrations in male SLE

belugas (adjusted $R^2 = 0.19$, $F_{1,18} = 5.43$, $p = 0.03$; Table 4 and Fig. 2). No candidate model stood out from the null model to explain the variation of other hormone levels in males nor females.

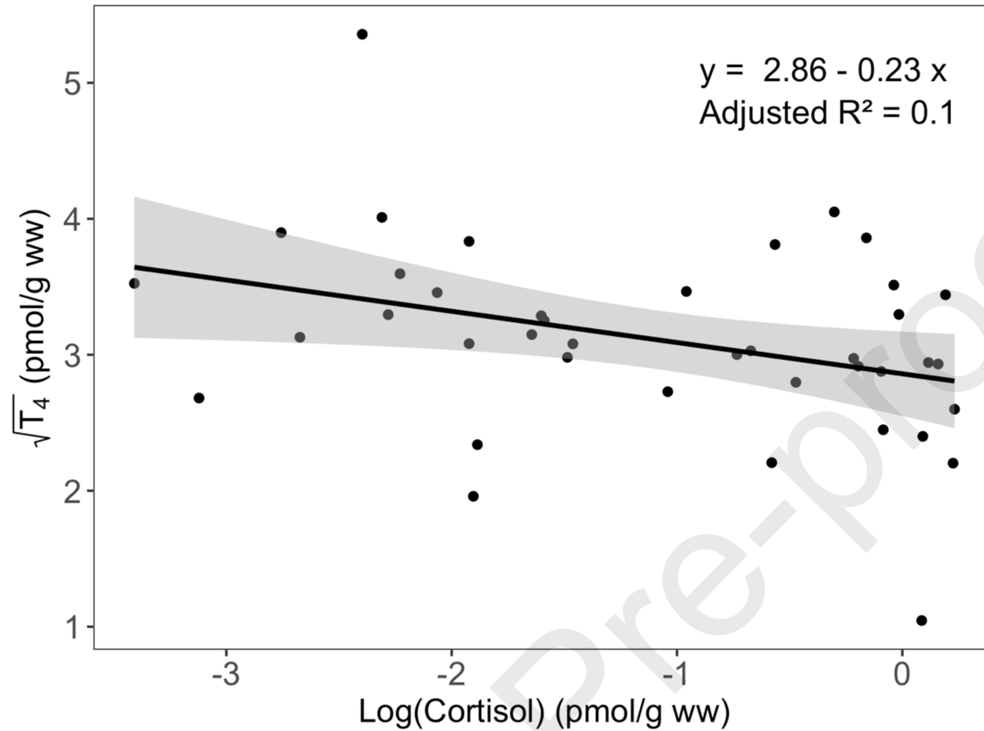


Figure 1. Linear regression between T_4 and cortisol levels in skin of SLE beluga males and females ($n = 40$). The shaded area represents 95% confidence interval.

Journal Pre-proofs

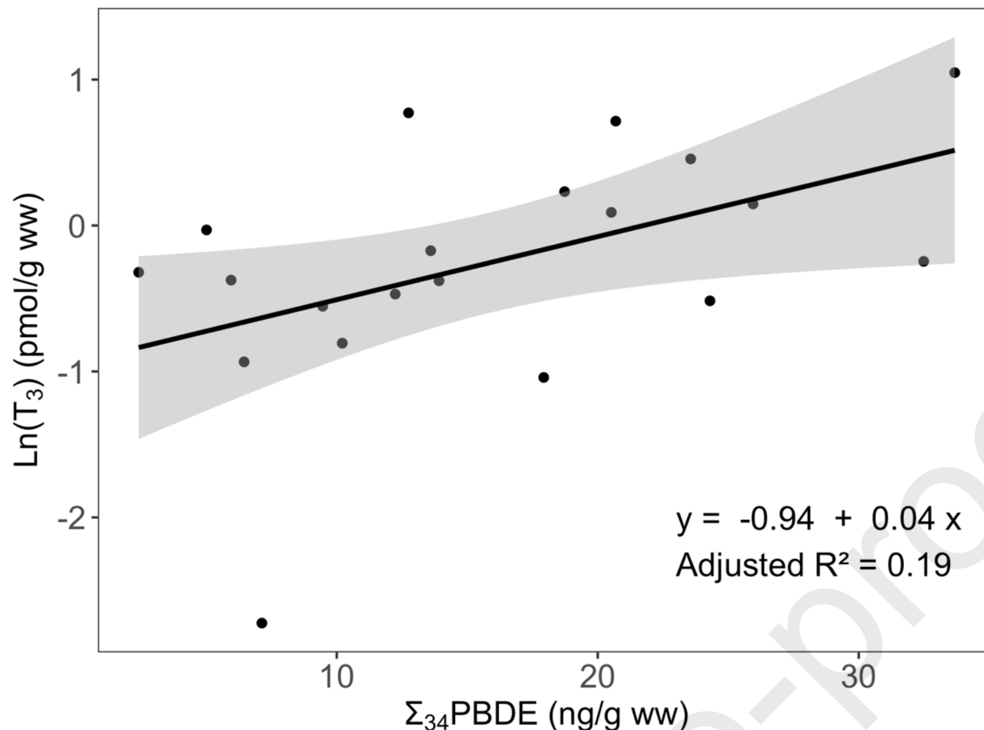


Figure 2. Linear regression between T_3 and Σ_{34}PBDE concentrations in skin of male SLE belugas (*n* = 20). The shaded area represents 95% confidence interval.

4. Discussion

4.1. Skin as an alternative matrix for hormone analysis in belugas

Hormone-related studies in cetaceans have mainly focused on steroid hormones to assess stress response (cortisol) and reproductive status (estrogens, androgens, and progestogens) (e.g., Boggs et al., 2017; Loseto et al., 2018; Cates et al., 2019; Galligan et al., 2019, 2020). In contrast, thyroid hormone levels have been relatively understudied despite being biomarkers of nutritional status and endocrine disruption (Tabuchi et al., 2006). Moreover, blood (plasma, serum) has traditionally been used to determine hormone levels in marine mammals. As such, plasma and serum levels of T_3, T_4, rT_3, and cortisol have previously been reported in other beluga populations (Norwegian Arctic, Alaska, Canadian Arctic, and captive belugas) (St. Aubin et al., 2001; Tryland et al., 2006; Schmitt et al., 2010; Spoon and Romano, 2012; Flower et al., 2015; Hansen et al., 2017; Loseto et al., 2018; Unal et al., 2018). However, ethical and technical considerations have prompted the development of less invasive methods than blood sampling for the study of hormones in free-ranging marine mammals, especially for populations at risk for which the capture of individuals cannot be considered. For example, steroid hormones such as cortisol have been successfully quantified in epidermal scrapes and blubber biopsies of several cetaceans (Bechshoft et al., 2015; Cates et al., 2019; Bechshoft et al., 2020; Wong et al., 2023). Thus, beluga skin biopsy represents a promising matrix for the study of moderately lipophilic hormones such as thyroid hormones as

Journal Pre-proofs

well as cortisol. Thyroid hormones and cortisol are both involved in energetic metabolism and beluga skin contains a relatively high lipid content (SLE beluga range: 2-17%). Moreover, thyroid hormones are involved in skin homeostasis and proliferation of epidermal cells in certain mammals (e.g., humans and rats) (Safer et al., 2004; Mancino et al., 2021) and are suspected of being involved in the process of molting in certain populations of belugas (St. Aubin et al., 1990). Furthermore, compared to immunoassays that have traditionally been used for hormone quantitation, LC-MS-based methods were shown to yield greater sensitivity, specificity, and accuracy (Cross and Hornshaw, 2016; Taylor et al., 2015). The present study showed that thyroid hormones (T_3 and T_4) and their deiodinated products (rT_3 and 3,5-T_2) as well as cortisol can be quantified using UPLC-MRM/MS from a small amount (~14 mg) of skin tissue. To our knowledge, this is the first report of thyroid hormones in skin of any cetaceans.

Comparisons of thyroid hormone levels between plasma and skin collected from wild and traditionally harvested Arctic belugas (all males) showed that both thyroid hormones and cortisol can be quantified in these matrices. Specifically, levels of T_4 and T_4/T_3 level ratios were markedly higher in plasma than in skin, and this difference was notably greater for cortisol as observed in past studies of Arctic beluga blubber and plasma (Loseto et al., 2018). These cortisol, T_4 and T_4/T_3 levels in plasma were expected, as elevated cortisol can be attributed to the acute stress related to the pursuit and harvest of these animals, while high T_4 in plasma versus T_3 reflects the T_4 conversion to T_3 as further deiodination takes place in peripheral tissue cells. However, levels of T_3, T_4, rT_3, and cortisol in skin of Arctic belugas were not related to those in plasma (no statistical analysis was performed due to low sample size). Thus, hormone levels determined in skin should be interpreted with caution as they do not represent the circulating thyroid hormone and cortisol status of the animals at the time of capture. A study on captive belugas found that the time lag between a stress event and the cortisol signal in outer epidermal scrapes was of 68 to 72 days, which is similar to the epidermal turnover rate in belugas (Wong et al., 2023). Further investigation is required to determine the relationships between thyroid hormone and cortisol levels in skin tissues and plasma as the sample size of our study was not large enough to draw any firm conclusions.

Previous studies on thyroid hormones in belugas have reported T_3, T_4, and rT_3 levels in serum and plasma only and were determined using immunoassays (Flower et al., 2015; Hansen et al., 2017), thus complicating comparisons with those measured in skin biopsies of present SLE belugas. Regardless, the most abundant thyroid hormone determined in SLE beluga skin was T_4, which reflects the fact that thyroid hormones in mammals are synthesized and released into the circulation primarily in the form of T_4. The high sensitivity of the UPLC-MRM/MS-based method also allowed for the quantification of the deiodinated metabolite 3,5-T_2, which is, to our knowledge, reported here in belugas for the first time. The 3,5-T_2 is weakly bioactive and was shown to have a role in energy metabolism in rodents (Senese et al., 2018). There were no significant differences between male and female SLE belugas for T_3, T_4, rT_3, 3,5-T_2, nor cortisol levels in skin. However, the T_4/T_3 level ratios were significantly lower in females compared to males. Contradictory information has been reported regarding the influence of sex on thyroid hormone status in belugas. For instance, Hansen et al. (2017) and Villanger et al. (2011) reported no difference between males

and females for the levels of T_3, T_4 and rT_3 in serum of belugas from the Norwegian Arctic (Svalbard), although the authors suggested that this lack of difference might be due to the low sample size. Conversely, significantly greater levels of circulating T_4 were measured in adult male belugas from the Canadian Arctic (St. Aubin et al., 2001) and from captive individuals under human care (Flower et al., 2015) compared to females.

4.2. Organohalogen levels in SLE belugas

Organohalogen concentrations in SLE beluga biopsies have previously been measured by Simond et al. (2019, 2020) in the innermost layer of skin, which consists of dermis and a small amount of blubber. By comparison, organohalogens in the present SLE beluga biopsies were measured in the epidermis and dermis layer. It has been shown that organohalogen concentrations may vary with depth in beluga skin and blubber tissues due to stratification (Krahn et al., 2004), which makes any comparisons between tissues approximative. For instance, concentrations of Σ_{40}PCB and Σ_{34}PBDE determined in present SLE beluga males were approximately 5- and 9-fold lower on a wet weight basis, respectively, than those reported by Simond et al. (2020) for dermis/blubber samples collected 3-4 years earlier (2015-2016). Nevertheless, consistently with findings reported by Simond et al. (2020), Σ_{40}PCB measured in the present study were the most abundant contaminant class in SLE beluga skin despite their ban in Canada in the late 1970s.

Concentrations of organohalogens are regularly reported to differ between sexes of marine mammals due to maternal transfer through lactation and the placenta, as also documented in belugas (Desforges et al., 2012; Cadieux et al., 2016). However, in the present study, no difference was found for any organohalogen concentrations between males and females. As such, Bernier-Graveline et al. (2021) similarly found no difference between males and females for the concentrations of Σ_{35}PBDE in blubber of SLE beluga carcasses recovered between 1998 and 2016, whereas Σ_{40}PCB and Σ_{25}OCP concentrations were significantly higher in males compared to females. This lack of differences in organohalogen concentrations in skin between males and females in the present study could be partially explained by the fact that sampling of females observed with a calf (and likely lactating) was avoided for ethical reasons.

4.3. Associations between thyroid hormones, cortisol, and organohalogens

We found that cortisol in SLE belugas was a weak predictor for the skin levels of T_4, rT_3, 3,5-T_2, and T_4/T_3 based on linear models ranked using AICc. Evidence of interactions (crosstalk) between the stress and thyroid axes have been reported in mammals (reviewed by Thambirajah et al., 2022). In fact, regulation through the stress axis shares a unique linkage with the thyroid axis in some vertebrates because corticotropin-releasing hormone (CRH) regulates both axes. As such, perturbation of CRH expression is predicted to translate into strong downstream effects in both the stress and thyroid axes (De Groef et al., 2006). For example, acute stress in adult male rats resulted in prolonged increase in glucocorticoid (corticosterone) secretion, and this was associated with a

Journal Pre-proofs

reduction and disruption of circadian changes in circulating thyroid stimulating hormone (TSH) (Martí et al., 1996). As such, chronic stress elevating cortisol levels (e.g., noise pollution, maritime traffic) in SLE belugas may render them more sensitive to adverse effects of organohalogens (or any other contaminants) acting via specific thyroid disrupting mechanisms (Thambirajah et al., 2022).

Concentrations of T_3 in skin were positively associated with those of Σ_{34}PBDE in male SLE belugas, whereas no such association was observed in females. These results suggest that PBDEs could potentially interfere with the regulation of thyroid hormones (T_3) in skin of SLE belugas. Studies investigating the associations between thyroid hormone levels and organohalogen exposure in marine mammals have used matrices that differ from the present study (e.g., plasma and blubber) and have reported conflicting results. A few studies on marine mammals have reported positive associations between tissue contaminant concentrations and thyroid hormone levels. For instance, in harbor seals (*Phoca vitulina*) sampled in the United Kingdom, circulating levels of T_3 were found to positively correlate with blubber concentrations of PCBs and PBDEs (Hall and Thomas, 2007). However, our results are in contrast with several reports of negative associations between thyroid hormone levels and organohalogen concentrations in marine mammals including belugas (Tabuchi et al., 2006; Gabrielsen et al., 2011; Villanger et al., 2011). In the study by Villanger et al. (2011), blubber concentrations of PBDEs (BDE-28, -47, -99, -100, and -154), PCBs (CB-105), and OCPs (HCB) were negatively correlated with circulating levels of T_4 and T_3 in Svalbard belugas. Again, the use of skin as an alternative matrix to plasma could also partly explain why the results of the present study differed from those of previous studies, potentially also implying different mechanisms of toxicity targeting the thyroid axis in these matrices.

HFRs (mainly PBDEs) and several other organohalogens were reported to disrupt thyroid hormone signaling and homeostasis through several target sites because of their structural similarities with these hormones. For instance, in SLE beluga skin biopsy, Simond et al. (2019) found a negative correlation between transcription levels of the gene encoding for the thyroid hormone receptor beta (THRB) in epidermis and concentrations of the HFR Dechlorane-604 component B (a debrominated product of Dechlorane-604) in dermis/blubber. These authors further reported positive correlations between transcription levels of the gene encoding for iodothyronine deiodinase type 2 (DIO2) in epidermis and certain organochlorines (PCBs, *p,p′*-DDE, *trans*-nonachlor, and HCB) in dermis/blubber. Since DIO2 catalyzes the transformation of T_4 to T_3, the positive association observed between PBDE concentrations and T_3 levels in present SLE beluga males could potentially stem, at least in part, from the perturbation of iodothyronine deiodinase activity in skin. In contrast to males, there was no association between PBDE concentrations and T_3 levels in females. This difference may be due to confounding factors affecting sexes differently, such as potential interactions between sex and thyroid hormones (and\or cortisol). The metabolic and biotransformation capacity towards PBDEs and other organohalogens may also differ between sexes in belugas (McKinney et al., 2004), although further research is needed to explore this hypothesis.

The assessment of endocrine disruption in wildlife is challenging because of the potential roles of several confounding factors that were not controlled for in the present study, such as other unmeasured contaminants, age, and reproductive and nutritional status (Flower et al., 2015). The associations found in our study may have been influenced by other contaminants. For example, contaminants of emerging concern such as the poly- and perfluoroalkyl substances and industrial antioxidants and UV absorbents have been detected in SLE belugas, and whether these can act as endocrine disruptors in this population should be investigated. Age can also influence both hormone levels and contaminant load in belugas. For example, in free-ranging belugas (Eastern Chukchi Sea and Bristol Bay, coast of Alaska, USA), juveniles exhibited higher serum T_3 and T_4 levels than adults (Flower et al., 2015). Additionally, cortisol levels in blubber were also found to increase with age in a study on Canadian Arctic belugas (Loseto et al., 2018). Furthermore, age can influence organohalogen concentrations in tissues of belugas. Older adult belugas generally accumulate higher concentrations of PBDEs, PCBs, and several OCPs (e.g., DDT, HCB, and Mirex) and PCBs (Martineau et al., 1987; Villanger et al., 2011; Lebeuf et al., 2014; Cadieux et al., 2016; Simond et al. 2017). Associations between thyroid hormones and contaminant exposure may be influenced by age as highlighted in a study on Faroese pilot whales (Hoydal et al., 2016). In the present study, only adult belugas were sampled, although their age was unknown as age determination typically involves the examination of tooth growth layers, which could not be performed. Pregnancy status may also influence thyroid hormone levels and contaminant concentrations (through placental transfer of contaminants to the fetus) in marine mammals (Desforges *et al.*, 2012, West et al., 2014). Though, sampling of females with calves was avoided, the reproductive status of females could not be assessed in our study.

Thyroid hormones were reported to be useful biomarkers of nutritional status in marine mammals (reviewed by Behringer et al., 2018). In Southern Resident killer whales, fecal T_3 concentrations decreased in response to nutritional stress, and low prey availability was associated with pregnancy failure (Wasser et al., 2017). Besides, in periods of nutritional stress, belugas may be more vulnerable to adverse effects of contaminants as their concentrations increase during lipid mobilization (Bernier-Graveline et al., 2021). As a previous study suggested that SLE belugas are facing a deterioration in energy reserves, nutritional stress should be investigated as a risk factor for endocrine disruption as well as dystocia and perinatal mortality in SLE belugas (Lair et al., 2016, Bernier-Graveline et al., 2021).

5. Conclusions

This study reported for the first time the levels of thyroid hormones in skin of a free-ranging marine mammal species, demonstrating that biopsy sampling represents a useful and less invasive technique to simultaneously determine levels of contaminants as well as thyroid hormones and cortisol in cetaceans. It was previously suggested that exposure to high levels of organohalogens could play a role in the increased number of parturition-related mortality in the SLE beluga population. The positive correlation found between skin PBDE and T_3 levels in SLE beluga males suggests that these contaminants could potentially act as endocrine disruptors (e.g., via perturbation of iodothyronine deiodinases), although it does not support our hypothesis of decreased thyroid

hormone levels in skin of this highly contaminant-exposed beluga population. Nevertheless, our results suggest that a combination of stress (i.e., elevated skin cortisol levels) along with organohalogen exposure (e.g., PBDEs and PCBs) may, at least in part, be associated with observed changes in thyroid hormone levels in skin potentially via crosstalk between the stress and thyroid axes (Thambirajah et al., 2022). However, these results must be interpreted with caution since no cause-effect relationship can be established from these associations. In fact, many confounding variables were not controlled for (e.g., age, nutritional status, other unmeasured contaminants, etc.). Further research should focus on the potentially interacting roles of chronic stress and contaminant exposure on thyroid hormone variations to provide a better understanding of the potential mechanisms of action of thyroid disruption in the endangered and declining SLE beluga population.

Acknowledgements

This research was supported primarily by the Fisheries and Oceans (DFO) Canada's Whale Science for Tomorrow program and the Natural Sciences and Engineering Research Council of Canada (NSERC). We acknowledge the contributions of A. Bernier-Graveline (Université du Québec à Montréal) for assistance with fieldwork, M. Moisan (GREMM) and his team for biopsy sampling, L. Wang (Université du Québec à Montréal) for HFR analysis, and Dr. J. Han (University of Victoria Genome BC Proteomics Centre) for thyroid and steroid hormone analysis. We are grateful to the Hendrickson Island beluga sampling program for providing comparative tissues for this study supported by the Tuktoyaktuk Hunters and Trappers Committee, the Fisheries Joint Management Committee, and Fisheries and Oceans Canada. We further thank A. Elliot and J. Brewster for lab support and review of analytical methods.

References

Barrett, H., Du, X., Houde, M., Lair, S., Verreault, J. & Peng, H. (2021). Suspect and Nontarget Screening Revealed Class-Specific Temporal Trends (2000-2017) of Poly- A nd Perfluoroalkyl Substances in St. Lawrence Beluga Whales. *Environmental Science and Technology*, *55*(3), 1659-1671. https://doi.org/10.1021/ACS.EST.0C05957/SUPPL_FILE/ES0C05957_SI_001.PDF

Bechshoft, T., Wright, A. J., Weisser, J. J., Teilmann, J., Dietz, R., Hansen, M., Björklund, E. & Styrishave, B. (2015). Developing a new research tool for use in free-ranging cetaceans: recovering cortisol from harbour porpoise skin. *Conservation Physiology*, *3*(1). https://doi.org/10.1093/CONPHYS/COV016

Bechshoft, T., Wright, A. J., Styrishave, B. & Houser, D. (2020). Measuring and validating concentrations of steroid hormones in the skin of bottlenose dolphins (*Tursiops truncatus*). *Conservation Physiology*, *8*(1). https://doi.org/10.1093/CONPHYS/COAA032

Behringer, V., Deimel, C., Hohmann, G., Negrey, J., Schaebs, F. S. & Deschner, T. (2018). Applications for non-invasive thyroid hormone measurements in mammalian ecology, growth, and maintenance. *Hormones and Behavior*, *105*, 66-85. https://doi.org/10.1016/J.YHBEH.2018.07.011

Bernier-Graveline, A., Lesage, V., Cabrol, J., Lair, S., Michaud, R., Rosabal, M. & Verreault, J. (2021). Lipid metabolites as indicators of body condition in highly contaminant-exposed belugas from the endangered St. Lawrence Estuary population (Canada). *Environmental Research*, *192*, 110272. https://doi.org/10.1016/J.ENVRES.2020.110272

Blouin, K., Malaisé, F., Verreault, J., Lair, S. & Lu, Z. (2022). Occurrence and temporal trends of industrial antioxidants and UV absorbents in the endangered St. Lawrence Estuary beluga whale (*Delphinapterus leucas*). *Science of The Total Environment*, *842*, 156635. https://doi.org/10.1016/J.SCITOTENV.2022.156635

Boggs, A. S. P., Schock, T. B., Schwacke, L. H., Galligan, T. M., Morey, J. S., Mcfee, W. E. & Kucklick, J. R. (2017). Rapid and reliable steroid hormone profiling in *Tursiops truncatus* blubber using liquid chromatography tandem mass spectrometry (LC-MS/MS). *Analytical and bioanalytical chemistry, 409(21), 5019-5029*. https://doi.org/10.1007/s00216-017-0446-z

Cadieux, M. A., Muir, D. C. G., Béland, P. & Hickie, B. E. (2016). Lactational Transfer of Polychlorinated-Biphenyls (PCBs) and Other Organochlorines in St. Lawrence Beluga Whales (*Delphinapterus leucas*). *Archives of Environmental Contamination and Toxicology*, *70*(1), 169-179. https://doi.org/10.1007/s00244-015-0223-y

Cates, K. A., Atkinson, S., Gabriele, C. M., Pack, A. A., Straley, J. M. & Yin, S. (2019). Testosterone trends within and across seasons in male humpback whales (*Megaptera novaeangliae*) from Hawaii and Alaska. *General and Comparative Endocrinology*. https://doi.org/10.1016/j.ygcen.2019.03.013

Cross, T. G. & Hornshaw, M. P. (2016). Can LC and LC-MS ever replace immunoassays? *Journal of Applied Bioanalysis*, *2*(4). https://doi.org/10.17145/jab.16.015

Desforges, J.-P.W., Ross, P.S. and Loseto, L.L. (2012), Transplacental transfer of polychlorinated biphenyls and polybrominated diphenyl ethers in arctic beluga whales (Delphinapterus leucas). Environmental Toxicology and Chemistry, 31: 296-300. https://doi.org/10.1002/etc.750

De Groef, B., Van der Geyten, S., Darras, V.M., Kühn, E.R. (2006). Role of corticotropin releasing hormone as a thyrotropin-releasing factor in non-mammalian vertebrates. Gen. Comp. Endocrinol. 146, 62–68. DOI: 10.1016/j.ygcen.2005.10.014

Fisheries and Oceans Canada (2024). Action Plan for the Beluga Whale (Delphinapterus leucas), St. Lawrence Population, in Canada. Series of Action Plans of the Species at Risk Act. Fisheries and Oceans Canada, Ottawa, Canada.

Flower, J. E., Allender, M. C., Giovanelli, R. P., Summers, S. D., Spoon, T. R., Leger, J. A. st., Goertz, C. E. C., Dunn, J. L., Romano, T. A., Hobbs, R. C. & Tuttle, A. D. (2015). Circulating concentrations of thyroid hormone in beluga whales (*delphinapterus leucas*): influence of age, sex, and season. *Journal of Zoo and Wildlife Medicine*, *46*(3), 456-467. https://doi.org/10.1638/2014-0127.1

Gabrielsen, K. M., Villanger, G. D., Lie, E., Karimi, M., Lydersen, C., Kovacs, K. M. & Jenssen, B. M. (2011). Levels and patterns of hydroxylated polychlorinated biphenyls (OH-PCBs) and their associations with thyroid hormones in hooded seal (*Cystophora cristata*) mother-pup pairs. *Aquatic Toxicology*, *105*(3-4), 482-491. https://doi.org/10.1016/j.aquatox.2011.08.003

Galligan, T. M., Balmer, B. C., Schwacke, L. H., Bolton, J. L., Quigley, B. M., Rosel, P. E., Ylitalo, G. M. & Boggs, A. S. P. (2019). Examining the relationships between blubber steroid hormones and persistent organic pollutants in common bottlenose dolphins. *Environmental Pollution*, *249*, 982-991. https://doi.org/10.1016/J.ENVPOL.2019.03.083

Galligan, T. M., Boggs, A. S. P., Balmer, B. C., Rowles, T., Smith, C. R., Townsend, F., Wells, R. S., Kellar, N. M., Zolman, E. S. & Schwacke, L. H. (2020). Blubber steroid hormone profiles as indicators of physiological state in free-ranging common bottlenose dolphins (*Tursiops truncatus*). *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *239*, 110583. https://doi.org/10.1016/J.CBPA.2019.110583

Journal Pre-proofs

Gilson, A., Syvanen, M., Levine, K. & Banks, J. (1998). Deer gender determination by polymerase chain reaction: Validation study and application to tissues, bloodstains, and hair forensic samples from California. *California Fish and Game*, *84*(4).

Hall, A. J. & Thomas, G. O. (2007). Polychlorinated biphenyls, DDT, polybrominated diphenylethers, and organic pesticides in United Kingdom harbor seals (*Phoca vitulina*) - Mixed exposures and thyroid homeostasis. *Environmental Toxicology and Chemistry*, 26(5), 851-861. https://doi.org/10.1897/06-310R.1

Hansen, M., Villanger, G. D., Bechshoft, T., Levin, M., Routti, H., Kovacs, K. M. & Lydersen, C. (2017). Circulating thyroid hormones and associated metabolites in white whales (*Delphinapterus leucas*) determined using isotope-dilution mass spectrometry. *Environmental Research*, *156*, 128-131. https://doi.org/10.1016/J.ENVRES.2017.03.027

Hoydal, K. S., Ciesielski, T. M., Borrell, A., Wasik, A., Letcher, R. J., Dam, M. & Jenssen, B. M. (2016). Relationships between concentrations of selected organohalogen contaminants and thyroid hormones and vitamins A, E and D in Faroese pilot whales. *Environmental Research*, *148*. 386-400. https://doi.org/10.1016/j.envres.2016.04.012

Hunt, K. E., Moore, M. J., Rolland, R. M., Kellar, N. M., Hall, A. J., Kershaw, J., Raverty, S. A., Davis, C. E., Yeates, L. C., Fauquier, D. A., Rowles, T. K., Kraus, S. D. & Cooke, S. (2013). Overcoming the challenges of studying conservation physiology in large whales: a review of available methods. *Conservation Physiology*, *1*(1). https://doi.org/10.1093/CONPHYS/COT006

Jugan, M. L., Levi, Y. & Blondeau, J. P. (2010). *Endocrine disruptors and thyroid hormone physiology*. *Biochemical Pharmacology*. *79*(7), 939-947. https://doi.org/10.1016/j.bcp.2009.11.006

Krahn, M. M., Herman, D. P., Ylitalo, G. M., Sloan, C. a, Burrows, D. G., Hobbs, R. C., Mahoney, B. a, Yanagida, G. K., Calambokidis, J. & Moore, S. E. (2004). Stratification of lipids, fatty acids and organochlorine contaminants in blubber of white whales and killer whales. *Journal of Cetacean Research and Managment*, *6*(2), 175-189.

Journal Pre-proofs

Lair, S., Measures, L. N. & Martineau, D. (2016). *Pathologic Findings and Trends in Mortality in the Beluga (*Delphinapterus leucas*) Population of the St Lawrence Estuary, Quebec, Canada, From 1983 to 2012*. *Veterinary Pathology*, *53*(1), 22-36. SAGE Publications Inc. https://doi.org/10.1177/0300985815604726

Lavoué, J., Joseph, L., Knott, P., Davies, H., Labrèche, F., Clerc, F., ... & Kirkham, T. (2019). Expostats: a Bayesian toolkit to aid the interpretation of occupational exposure measurements. Annals of work exposures and health, 63(3), 267-279. DOI: 10.1093/annweh/wxy100

Lebeuf, M., Measures, L., Noël, M., Raach, M. & Trottier, S. (2014). A twenty-one year temporal trend of persistent organic pollutants in St. Lawrence Estuary beluga, Canada. *Science of the Total Environment*, *485-486*(1), 377-386. https://doi.org/10.1016/j.scitotenv.2014.03.097

Loseto L, Pleskach K, Hoover C, Tomy GT, Desforges J-P, Halldorson T, Ross PS (2018). Cortisol levels in beluga whales (*Delphinapterus leucas*): Setting a benchmark for Marine Protected Area monitoring. Arctic Science. 4(3): 358-372. https://doi.org/10.1139/as-2017-0020

Mancino, G., Miro, C., di Cicco, E. & Dentice, M. (2021). Thyroid hormone action in epidermal development and homeostasis and its implications in the pathophysiology of the skin. *Journal of Endocrinological Investigation*, 44(8). https://doi.org/10.1007/s40618-020-01492-2

Martí, O., Gavald`a, A., Jolín, T., Armario, A., 1996. Acute stress attenuates but does not

abolish circadian rhythmicity of serum thyrotrophin and growth hormone in the rat.

Eur. J. Endocrinol. 135, 703–708. DOI: 10.1530/eje.0.1350703

Martineau, D., Béland, P., Desjardins, C. & Lagacé, A. (1987). Levels of organochlorine chemicals in tissues of beluga whales (*Delphinapterus leucas*) from the St. Lawrence Estuary, Québec, Canada. *Archives of Environmental Contamination and Toxicology*, *16*(2). https://doi.org/10.1007/BF01055795

Mazerolle, M. J. (2020). *AICcmodavg: Model Selection and Multimodel Inference Based on (Q)AIC(c)* (Version 2.3-1). R package.

Journal Pre-proofs

McKinney, M. A., Arukwe, A., de Guise, S., Martineau, D., Béland, P., Dallaire, A., Lair, S., Lebeuf, M., & Letcher, R. J. (2004). Characterization and profiling of hepatic cytochromes P450 and phase II xenobiotic-metabolizing enzymes in beluga whales (Delphinapterus leucas) from the St. Lawrence River Estuary and the Canadian Arctic. *Aquatic Toxicology*, *69*(1). https://doi.org/10.1016/j.aquatox.2004.04.010

McNabb, F. M. A. (1992). *Thyroid hormones*. Prentice Hall.

Mosnier, A., Doniol-Valcroze, T., Gosselin, J. F., Lesage, V., Measures, L. N. & Hammill, M. O. (2015). Insights into processes of population decline using an integrated population model: The case of the St. Lawrence Estuary beluga (*Delphinapterus leucas*). *Ecological Modelling*, *314*, 15-31. https://doi.org/10.1016/J.ECOLMODEL.2015.07.006

Pelt, A.C., 2011. Glucocorticoids: Effects, Action Mechanisms, and Therapeutic Uses.

Nova Science, Hauppauge, NY.

R Core Team. (2021). *R: A Language and Environment for Statistical Computing* (4.1.1). https://www.r-project.org/

Schmitt, T. L., St. Aubin, D. J., Schaefer, A. M., & Dunn, J. L. (2010). Baseline, diurnal variations, and stress-induced changes of stress hormones in three captive beluga whales, Delphinapterus leucas. Marine Mammal Science, 26(3), 635-647. https://doi.org/10.1111/j.1748-7692.2009.00366.x

Safer, J. D., Crawford, T. M. & Holick, M. F. (2004). A Role for Thyroid Hormone in Wound Healing through Keratin Gene Expression. *Endocrinology*, *145*(5), 2357-2361. https://doi.org/10.1210/EN.2003-1696

Senese, R., de Lange, P., Petito, G., Moreno, M., Goglia, F. & Lanni, A. (2018). 3,5-Diiodothyronine: A novel thyroid hormone metabolite and potent modulator of energy

Journal Pre-proofs

metabolism. *Frontiers in Endocrinology*, *9*(JUL), 427. https://doi.org/10.3389/FENDO.2018.00427/BIBTEX

Simond, A. E., Houde, M., Lesage, V. & Verreault, J. (2017). Temporal trends of PBDEs and emerging flame retardants in belugas from the St. Lawrence Estuary (Canada) and comparisons with minke whales and Canadian Arctic belugas. Environmental Research, 156, 494 504. https://doi.org/10.1016/j.envres.2017.03.058

Simond, A. E., Houde, M., Lesage, V., Michaud, R., Zbinden, D. & Verreault, J. (2019). Associations between organohalogen exposure and thyroid-and steroid-related gene responses in St. Lawrence Estuary belugas and minke whales. Marine Pollution Bulletin, 145, 174 184. https://doi.org/10.1016/j.marpolbul.2019.05.029

Simond, A. E., Houde, M., Lesage, V., Michaud, R. & Verreault, J. (2020). Metabolomic profiles of the endangered St. Lawrence Estuary beluga population and associations with organohalogen contaminants. *Science of the Total Environment*, *717*, 137204. https://doi.org/10.1016/j.scitotenv.2020.137204

Sonne, C., Siebert, U., Gonnsen, K., Desforges, J. P., Eulaers, I., Persson, S., Roos, A., Bäcklin, B. M., Kauhala, K., Tange Olsen, M., Harding, K. C., Treu, G., Galatius, A., Andersen-Ranberg, E., Gross, S., Lakemeyer, J., Lehnert, K., Lam, S. S., Peng, W. & Dietz, R. (2020). Health effects from contaminant exposure in Baltic Sea birds and marine mammals: A review. *Environment International*, *139*, 105725. https://doi.org/10.1016/J.ENVINT.2020.105725

Spoon, T. R., & Romano, T. A. (2012). Neuroimmunological response of beluga whales (*Delphinapterus leucas*) to translocation and a novel social environment. Brain, Behavior, and Immunity, 26(1), 122-131. DOI: 10.1016/j.bbi.2011.08.003

St. Aubin, D. J., Deguise, S., Richard, P. R., Smith, T. G. & Geraci, J. R. (2001). Hematology and plasma chemistry as indicators of health and ecological status in beluga whales, *Delphinapterus leucas*. *Arctic*, *54*(3), 317-331. https://doi.org/10.14430/arctic791

St. Aubin, D. J., Smith, T. G. & Geraci, J. R. (1990). Seasonal epidermal molt in beluga whales, Delphinapterus leucas. *Canadian Journal of Zoology*, *68*(2), 359 367. https://doi.org/10.1139/z90-051

Tabuchi, M., Veldhoen, N., Dangerfield, N., Jeffries, S., Helbing, C. C. & Ross, P. S. (2006). PCB-related alteration of thyroid hormones and thyroid hormone receptor gene expression in free-ranging harbor seals (*Phoca vitulina*). *Environmental Health Perspectives*, *114*(7), 1024-1031. https://doi.org/10.1289/ehp.8661

Taylor, A. E., Keevil, B. & Huhtaniemi, I. T. (2015). Mass spectrometry and immunoassay: how to measure steroid hormones today and tomorrow. *European journal of endocrinology*, *173*(2), D1-D12. https://doi.org/10.1530/EJE-15-0338

Thambirajah, A. A., Wade, M. G., Verreault, J., Buisine, N., Alves, V. A., Langlois, V. S. & Helbing, C. C. (2022). Disruption by stealth - Interference of endocrine disrupting chemicals on hormonal crosstalk with thyroid axis function in humans and other animals. *Environmental Research*, *203*. https://doi.org/10.1016/j.envres.2021.111906

Tryland M, Thoresen SI, Kovacs KM, Lydersen C. (2006) Serum chemistry of free-ranging white whales (Delphinapterus leucas) in Svalbard. Vet Clin Pathol. 35(2):199-203. doi: 10.1111/j.1939-165x.2006.tb00114.x

Unal, E., Goertz, C. E., Hobbs, R. C., Suydam, R., & Romano, T. (2018). Investigation of molecular biomarkers as potential indicators of health in wild belugas (Delphinapterus leucas). Marine Biology, 165(12), 182. https://doi.org/10.1007/s00227-018-3439-3

Villanger, G. D., Lydersen, C., Kovacs, K. M., Lie, E., Skaare, J. U. & Jenssen, B. M. (2011). Disruptive effects of persistent organohalogen contaminants on thyroid function in white whales (*Delphinapterus leucas*) from Svalbard. *Science of the Total Environment*, *409*(13), 2511-2524. https://doi.org/10.1016/j.scitotenv.2011.03.014

Wasser, S. K., Lundin, J. I., Ayres, K., Seely, E., Giles, D., Balcomb, K., Hempelmann, J., Parsons, K. & Booth, R. (2017). Population growth is limited by nutritional impacts on

Journal Pre-proofs

pregnancy success in endangered Southern Resident killer whales (*Orcinus orca*). *PLOS ONE*, *12*(6), e0179824. https://doi.org/10.1371/JOURNAL.PONE.0179824

West, K. L., Ramer, J., Brown, J. L., Sweeney, J., Hanahoe, E. M., Reidarson, T., Proudfoot, J. & Bergfelt, D. R. (2014). Thyroid hormone concentrations in relation to age, sex, pregnancy, and perinatal loss in bottlenose dolphins (*Tursiops truncatus*). *General and Comparative Endocrinology*, *197*, 73-81. DOI: 10.1016/j.ygcen.2013.11.021

Wong CH, Tsai MA, Ko FC, Wang JH, Xue YJ, Yang WC. (2023). Skin Cortisol and Acoustic Activity: Potential Tools to Evaluate Stress and Welfare in Captive Cetaceans. Animals (Basel). 13(9):1521. doi: 10.3390/ani13091521

Zoeller, R. T., Tan, S. W. & Tyl, R. W. (2007). General background on the hypothalamic-pituitary-thyroid (HPT) axis. *Critical Reviews in Toxicology*, *37*(1-2), 11-53. https://doi.org/10.1080/10408440601123446

Valérie Jolicoeur: Formal analysis, Investigation, Methodology, Writing – original draft; Magali Houde: Methodology, Supervision, Writing – review & editing; Lisa Loseto: Methodology, Writing – review & editing; Robert Michaud: Methodology, Writing – review & editing; Jonathan Verreault: Conceptualization, Funding acquisition, Project administration, Supervision, Writing – original draft, review & editing.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

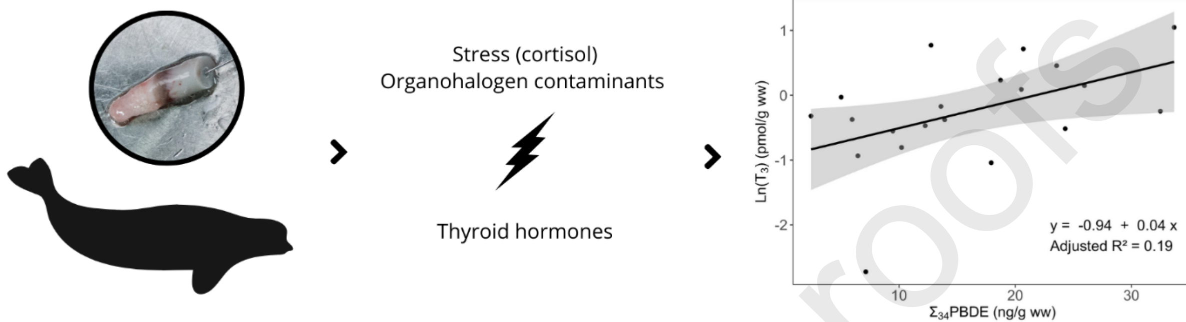
☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Highlights

- The endangered St. Lawrence Estuary beluga population is highly contaminated
- Cortisol and thyroid hormones were analyzed in beluga skin by UPLC-MRM/MS

Journal Pre-proofs

- T_4 levels were negatively associated with those of cortisol in beluga skin
- T_3 levels were positively associated with PBDE concentrations in beluga males



Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Journal Pre-proofs