

# Serum thyroid hormone concentrations and thyroid histomorphology as biomarkers in bowhead whales (*Balaena mysticetus*)

Cheryl Rosa, Todd M. O'Hara, Paul F. Hoekstra, Kent R. Refsal, and John E. Blake

**Abstract:** Serum thyroid hormone (TH) concentrations have been used alone or with other measurements to assess health status or effects of toxicant exposure in marine mammals. Histological sections from thyroid glands of the bowhead whale (*Balaena mysticetus* L., 1758) were examined in conjunction with serological TH analyses. Serum was assayed for total and free triiodothyronine and total and free thyroxine via radioimmunoassay. Histomorphology of thyroid tissue was assessed by light microscopy and the utilization of an epithelial-follicular index (EFI). Age, sex, or season did not significantly affect serum TH levels. However, TH concentrations in pregnant or lactating females were found to be significantly lower than in the other sex and reproductive groups investigated. The EFI and epithelial height (EH) were greater in spring subadult and adult whales compared with those that were landed in the fall. No correlation was found between serum TH concentrations and serum, blubber, or liver levels of select polychlorinated biphenyl metabolites and organochlorine congeners examined. Low variability in concentrations of the serum THs across age, season, and sex and reproductive groups supports the existence of strong homeostatic mechanisms for maintaining TH concentrations in these presumably healthy animals. Departures from these ranges may indicate a disturbance in these regulatory mechanisms and may be a useful indication of toxicity or other health disorders.

**Résumé :** Les concentrations sériques de l'hormone thyroïdienne (TH) servent seules, ou en combinaison à d'autres mesures, à évaluer l'état de santé ou à déterminer les effets d'expositions à des substances toxiques chez les mammifères marins. Nous avons examiné des coupes histologiques de la thyroïde de la baleine franche boréale (*Balaena mysticetus* L., 1758) en conjonction avec des analyses de la TH sérique. Nous avons dosé la triiodothyronine totale et libre et la thyroxine totale et libre dans le sérum par test radioimmunologique. Nous avons évalué l'histomorphologie du tissu thyroïdien au microscope photonique à l'aide de l'indice épithélial-folliculaire (EFI). Ni l'âge, ni le sexe, ni la saison n'affectent significativement les concentrations de TH. Cependant, les concentrations de TH chez les femelles enceintes ou allaitantes sont significativement plus faibles que chez les groupes de l'autre sexe et d'état reproducteur différent. L'indice EFI et la hauteur de l'épithélium (EH) sont plus élevés chez les baleines subadultes et adultes du printemps que chez celles qui sont capturées à l'automne. Il n'y a pas de corrélation entre les concentrations sériques de TH et les dosages de certains métabolites sélectionnés des biphényls polychlorés et des congénères organochlorés examinés dans le sérum, le lard ou le foie. La faible variabilité des concentrations de TH du sérum en fonction de l'âge, de la saison et du sexe et groupe reproducteur laisse croire à l'existence d'importants mécanismes homéostatiques pour le maintien des concentrations de TH dans ces animaux qui sont présumés être en bonne santé. Tout écart hors de ces limites peut indiquer une perturbation de ces mécanismes régulateurs et peut servir d'indicateur de toxicité ou d'autres problèmes de santé.

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## Introduction

Serum thyroid hormone (TH) levels have been suggested as useful biomarkers of contaminant exposure and as surrogate measures of health in several species of marine and terrestrial mammals (Béland et al. 1993; Schumacher et al.

1993; De Guise et al. 1995; Rolland 2000). A useful biomarker should reliably measure a change induced by one or more contaminants (or class of compounds) in the biochemical or cellular components of a process, structure, or function (NRC 1989). Unlike a diagnostic test used to assess clinical disease, a biomarker must be sensitive enough to de-

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fect an early change that may eventually progress to overt clinical disease or significantly altered physiology, such as impaired immune function or reproduction. Most biomarkers do not possess the specificity to identify the particular agent or agents causing the measured change. Instead, they indicate that a change has occurred and that subsequent investigation is warranted; however, a good biomarker should help direct the diagnostic effort. Biomarkers can be separated into three broad groups: biomarkers of exposure, biomarkers of effect, and biomarkers of susceptibility (Arking 1991). The search for indicators of marine mammal health or toxicant exposure has identified many potential candidates, including cytochrome P450 isozyme induction (Hahn et al. 2000; Angell et al. 2004), C-reactive protein (Funke et al. 1997), vitamin A (Simms and Ross 2000), benzo(a)pyrene monooxygenase (BPMO) activity (Fossi et al. 2003), and serum THs (Schumacher et al. 1993; Hall et al. 1998, 2003).

THs are relatively easy to measure in most species, but reliable results depend on method validation. There is little published information available on cetacean serum TH levels and thyroid gland histomorphology. There are also few references describing the gross anatomical features of cetacean thyroid glands (Harrison 1969; Ridgeway and Patton 1971; Shimokawa et al. 2002). With few exceptions, mammalian thyroid histomorphology and ultrastructure exhibit such little variability that it would be difficult to differentiate between species using light or electron microscopy. In contrast to microscopic and ultrastructural anatomy, total and free serum TH reference ranges vary significantly from one mammalian species to another (Joasoo et al. 1975; Tomasi et al. 1998; Ortiz et al. 2000). Within a single species, many different factors such as season, nutritional status, reproductive state, contaminant load, and health condition can influence TH activity (Béland et al. 1993; Schumacher et al. 1993; De Guise et al. 1995; Rolland 2000; Bhagavan 2002).

In cetaceans, correlation between the variability in TH concentration and season may be influenced by factors such as water temperature, day length, reproduction or lactation, and feeding or fasting (St. Aubin and Geraci 1988, 1989; St. Aubin et al. 2001). Substantial variability in the function and histomorphology of thyroid glands that correlates with seasonal changes was first identified in beluga whales, *Delphinapterus leucas* (Pallas, 1776) (St. Aubin and Geraci 1989; St. Aubin et al. 2001).

THs are important in the hormonal regulation of seasonal breeders (Dahl et al. 1994; Viguié et al. 1999). Sex-related differences in TH concentration have been found in marine mammals, although results vary. Age- and sex-related differences in total thyroxine (T4) and triiodothyronine (T3) have been found in the beluga whale (St. Aubin et al. 2001), while no effect of sex on T4, T3, or thyroid hormone binding capacity (THBC) was found in bottlenose dolphins (*Tursiops truncatus* (Montagu, 1821)) as measured by radioimmunoassay (Greenwood and Barlow 1979). There are limited data on TH levels in pregnant wildlife and no published information for pregnant cetaceans found in the scientific literature.

Alterations in thyroid function and morphology have been induced by PCBs in experimental animals (Bryne et al. 1987; Brouwer et al. 1989) and have been related to the effects of organochlorines (OCs) on TH transport proteins,

receptors, and TH activity. Captive harbor seals (*Phoca vitulina* L., 1758) that were experimentally fed fish contaminated with PCBs exhibited reductions in serum tT4 and fT4 concentrations compared with seals that ingested fish from non-contaminated waters (Brouwer et al. 1989). Plasma T3 deficiency has also been associated with chlorinated hydrocarbon exposure in spotted seals (*Phoca largha* Pallas, 1811) and ribbon seals (*Histiophoca fasciata* (Zimmermann, 1783)) (Chiba et al. 2001). These findings are important to consider when evaluating marine mammal population health.

The bowhead whale (*Balaena mysticetus* L., 1758) is an endangered mysticete species that lives in Arctic and sub-arctic waters. Although the Bering – Chukchi – Beaufort Sea (BCBS) stock was commercially exploited to near extinction in the late 1800s, it is currently growing (George et al. 2004) and is an important subsistence species to residents of northern Alaska, Russia, and Canada. The BCBS stock undertakes a yearly migration between the Bering Sea region and the Beaufort Sea to reach the highly productive summer feeding grounds located there, providing the opportunity to obtain high-quality biological samples from landed whales in the spring and fall.

As part of a larger health assessment study, we analyzed serum TH concentrations (total T3 and T4, as well as free T3 and T4) in bowhead whales and compared these levels with their thyroid histomorphology. Our primary objective was to describe the relationship between thyroid function and histomorphology with consideration given to season, sex and reproductive stage, age, and OC concentrations in serum, blubber, and liver. We also describe the gross anatomy of the thyroid gland in the bowhead whale and provide data that will form a “baseline” for future evaluation of TH levels and thyroid histopathology in the species.

## Materials and methods

### Sample collection and processing

Thyroid tissue and blood samples were collected during the 1998–2002 spring and fall Inuit subsistence harvest in Barrow, Alaska. These sample collections were conducted with permission of the Barrow Whaling Captain's Association and the Alaska Eskimo Whaling Commission (AEWC) through the Department of Wildlife Management (North Slope Borough, Alaska) under the purview of a National Oceanic and Atmospheric Administration (NOAA) permit (Nos. 932-1489-00 and 932-1489-03 for the Marine Mammal Health and Stranding Program (MMHSRP) issued to Dr. Teri Rowles). Blood was collected into untreated evacuated red top tubes (Vacutainer/BD, Franklin Lakes, New Jersey) approximately 2–14 h post mortem from the palatal sinus of 61 bowhead whales and 1 fetus. The blood was allowed to clot and was centrifuged for 10 min at 3500g within 4–6 h of collection (blood was kept at 5 °C during the holding period). The serum was transferred by pipette to a 5 mL plastic culture tube and frozen at –20 °C, then archived at –80 °C until thawed for hormone analyses.

A central portion of the thyroid gland ( $n = 24$ ) was preserved in 10% buffered formalin within 3–14 h of death. Not all whales had thyroid glands that were appropriate for fixation secondary to autolysis or freeze artifact. After fixa-

tion, 5 µm sections embedded in paraffin were stained with hematoxylin and eosin. Digital photomicrographs and measurements were taken using a Zeiss AxioCam camera and AxioVision software version 3.2 (Carl Zeiss, Inc., Thornwood, New York).

### Hormone analyses

All serum analyses were performed at the Diagnostic Center for Population and Animal Health, Michigan State University, East Lansing. Total thyroxine (T4) was measured with a commercially available solid-phase radioimmunoassay (RIA) kit (Clinical Assays Gammacoat M Total T4 <sup>125</sup>I RIA kit; DiaSorin Inc, Stillwater, Minnesota). Specificity data from the manufacturer identified 92% cross-reactivity with D-thyroxine, 2.1% cross-reactivity with D- and L-triiodothyronine (T3), and <0.1% cross-reactivity with other iodothyronines. The reagents were prepared as described in the manufacturer's protocol. Modifications were made to the protocol to enhance the analytical sensitivity of the assay. The volume of sample or standard was increased from 10 to 25 µL for all samples analyzed. A "lower" standard was made by mixing equal volumes of 0 and 13 nmol/L standards. The high standard (257 nmol/L) included in the kit was discarded, leaving the 156 nmol/L standard as the highest in the curve. After pipetting the sample or standard and 1 mL of radioligand solution into antibody-coated tubes, the assay was incubated at room temperature (~22 °C) for 3 h. The sensitivity assay, defined as the concentration of T4 at 90% specific binding, was 3 nmol/L (data from 10 assays). When L-thyroxine was added to aliquots of a pool of bowhead whale serum to create increases of 26 and 52 nmol/L, 93% and 81% of added T4 were measured in the respective assays. A pool of bowhead whale serum with a T4 concentration of 94 nmol/L was diluted with "0" standard at 50% and 25% dilutions. Assay of these diluted samples yielded 91% and 99% recoveries, respectively, when corrected for dilution. Intra-assay repeatability was determined in a pool of bowhead whale serum with a T4 concentration of 94 nmol/L. The respective intra-assay and inter-assay coefficients of variation (CVs) for 10 replicates of this pool were 0.038 and 0.024.

Total triiodothyronine (T3) was measured by a charcoal separation RIA, whose procedures and modifications have been previously described in Refsal et al. (1984) and Panciera et al. (1990). The sensitivity of the assay, described as the T3 concentration at 90% specific binding was 0.26 nmol/L (data from 10 assays). When T3 was added to aliquots of a pool of bowhead whale serum to produce increases of 1.5 and 3.0 nmol/L, 113% and 114% of added T3 were measured in the assay, respectively. A pool of bowhead whale serum with a T3 concentration of 1.03 nmol/L was diluted to 50% and 25% dilutions in "0" standard, with 107% and 158% recoveries, respectively, when corrected for dilution. Assay repeatability was determined with the same pool of bowhead whale serum. The respective intra-assay and inter-assay CVs for 10 replicates of this pool were 0.091 and 0.097.

Assays for free T4 in bowhead whale sera were done using materials and reagents in a commercially available kit (Free T4 by equilibrium dialysis; Nichols Institute Diagnostics, San Clemente, California). The procedures for equilibrium dialysis and RIA of T4 in dialysate were done

following the manufacturer's protocol. The manufacturer reported <0.044% cross-reactivity of other iodothyronines in the RIA. The sensitivity of the assay, defined as the concentration of free T4 at 90% specific binding, was 1.8 pmol/L (mean of 10 assays). Estimates of dilutional parallelism and recovery were made with dialysates of bowhead whale serum. When a pool of dialysate with a free T4 concentration of 29 pmol/L was diluted with dialysate buffer by 50% and 25%, 100% and 158% of expected amounts of free T4 were measured in the assay, respectively. When aliquots of T4 equivalent to 5, 12, 29, and 66 pmol/L were added to the same pool of bowhead whale dialysate, 104%, 102%, 113%, and 108% of the respective added T4 were measured in the assay. Estimates of repeatability were also made with the same pool of bowhead whale serum. For 10 replicates of each pool, the respective intra-assay and inter-assay CVs were 0.089 and 0.148.

Free T3 was measured using a commercially available solid-phase RIA based on competition of endogenous free T3 with a <sup>125</sup>I-labeled triiodothyronine derivative (Clinical Assays GammaCoat Free T3 <sup>125</sup>I RIA kit; DiaSorin Inc, Stillwater, Minnesota). The kit protocol from the manufacturer described 100% antibody cross-reactivity with L-triiodothyronine and <0.2% cross-reactivity with all other iodothyronines tested. The volumes of samples, standards, and radioligand were used according to the manufacturer's protocol. A modification to the assay procedure was to extend the duration of incubation from 90 min to 3 h in a 37 °C water bath. This change was done to ensure equilibration of maximal binding for assay runs that consisted of a standard curve and 53 samples. The sensitivity of the assay, defined as the concentration of free T3 at 90% specific binding, was 1.2 pmol/L (based on data from 10 assays). In analog-based RIA for free T3, there are multiple binding interactions between the endogenous hormone, the T3 derivative, assay antibody, and endogenous binding proteins. In this circumstance, assessment of dilutional parallelism and recovery is not possible. For a bowhead whale serum pool with a free T3 concentration of 2.8 pmol/L, the respective intra-assay and inter-assay CVs were 0.044 and 0.089 (10 replicates).

### Histomorphometric analysis

An epithelial-follicular index (EFI) was calculated with the aid of a technique adapted from Sidor (1971) using digital images (*n* = 24 whales). One hundred thyroid follicles per whale were selected at random for measurement using a labeled grid (Lovin Field Finder, Gurley Precision Instruments, Troy, New York). Samples showing evidence of extensive autolysis, resulting in cell or follicle distortion, were not measured. A follicular length (FL) was taken between the farthest distant two points in the follicle. A follicular width (FW) was then measured perpendicular to the initial FL measurement. Four measurements of epithelial height (EH) per follicle were then made at each of these four extremities (from the innermost aspect to the outermost aspect of the follicle lining cell). The following formula was used to calculate follicle size (FS):

$$FS = \frac{FL + FW}{2}$$

Once FS was calculated, the EFI was determined as

$$\text{EFI} = \frac{\text{EH} \times 100}{\text{FS}}$$

### Aging

The ages of 35 whales were designated juvenile, subadult, or adult, based on baleen stable isotope analysis of carbon ( $\delta^{13}\text{C}$ ) signature and aspartic acid racemization of eye lens nuclei (George et al. 1999; Lubetkin et al. 2004; Rosa et al. 2004). Juveniles were 1- to 3-year-olds that experienced an accelerated period of growth; subadults were 3-year-olds to approximately 22-year-olds in males and 25-year-olds in females (sexually immature); and adults were >22-year-olds in males and >25-year-olds in females (sexually mature) (George et al. 1999, 2004); Lubetkin et al. 2004; O'Hara et al. 2002; Rosa et al. 2004).

Not all whales were aged via laboratory methods secondary to tissue availability and the high confidence with which whales could be placed into an appropriate age group (i.e., juvenile or adult). The remaining 26 whales were categorized using a combination of body length, baleen length, and gonadal size or development (George et al. 1999; Tarp-ley and Hillmann 1999; O'Hara et al. 2002).

### Persistent organochlorine (OC) analyses

The OC data set used in this study was previously reported (Hoekstra et al. 2002, 2003) and many OCs are known to have a relatively high affinity for the T4 receptors on transthyretin (TTR) or impair thyroid function (Ishihara et al. 2003). Potential relationships or interactions between concentrations of various OCs, including 40 PCB congeners as well as selected hydroxylated PCB (OH-PCB) and methylsulfone-containing PCB (MeSO<sub>2</sub>-PCB) metabolites that occur in greatest mean concentrations in serum, blubber, and liver, were selected for multivariate analysis.<sup>4</sup> These OCs were detected at relatively low levels in most whales.

### Statistical analysis

Data are presented as means  $\pm$  SD. Thyroid data (tT3, tT4, fT3, fT4) were analyzed by a three-way analysis of variance (GLM, general linear model) with interaction terms (age class  $\times$  sex  $\times$  season) and Student's *t* test (EFI and follicle size comparisons). Multivariate methods (canonical correlation) were used to determine thyroid hormone and OC relationships. Wilks's  $\lambda$  was used to test the significance of the first canonical correlation and a likelihood ratio test was used to test the linear relationship between the canonical variables.

All statistical analyses were performed on the SAS<sup>®</sup> operating system (SAS Institute Inc., Cary, North Carolina). Values were considered significantly different at  $P < 0.05$ .

## Results

The bowhead whale thyroid gland is located along the ventral trachea, cranial to where the trachea bifurcates into

primary bronchi. Right and left lobes are easily discriminated and no isthmus was observed between the two lobes. The gland consisted of follicles of variable size that are lined by simple epithelium of variable height. Qualitatively, thyroid follicles from the thyroid glands of bowhead whales landed in the spring tended to be small, with a diminished amount of colloid and tall cuboidal to columnar epithelial lining cells containing large, open-faced nuclei. The typical appearance of thyroid glands collected from adults and subadults in the fall was characterized by colloid-distended follicles lined with attenuated epithelial cells.

The serum thyroid hormone results are summarized in Table 1. There was no significant difference in thyroid hormone levels found between seasons, sexes, or among age (length) groups.

Pregnant and lactating females ( $n = 5$ ), however, had significantly lower tT4 and fT4 than nonpregnant adult females ( $n = 7$ ) and other age and sex classes ( $n = 49$ ) (Table 2). Data from one fetal serum sample (not included in this data set) indicate that near-term fetal thyroid levels (tT4 = 136 nmol/L, tT3 = 3.1 nmol/L, fT4 = 53 pmol/L, fT3 = 9.8 pmol/L) are high compared with the means for juvenile, subadult, and adult whales.

Subadult and adult follicle size was significantly larger in fall versus spring (fall:  $307.56 \pm 190.73$ ; spring:  $67.68 \pm 18.15$ ;  $P = 0.036$ ). There was no difference between the sexes in any age group.

When all age groups of whales were considered together (juveniles, subadults, adults), there was no significant difference found between spring and fall follicle size ( $P = 0.343$ ). The size distribution of follicles in juveniles during the fall appeared similar to those of all age groups considered together during the spring. However, when adults and subadults were considered (juveniles omitted), the fall distribution (subadults and adults only) was different from the spring distribution, with more of the larger size classes of follicles represented. A photomicrographic comparison of spring and fall thyroid histology is shown in Fig. 1.

There were no significant differences found in the EFI values by three-way ANOVA when all age classes were analyzed together ( $P = 0.65$ ). For subadult and adult whales (juveniles omitted owing to their maintenance of active epithelial cells in spring and fall), those harvested in the spring have significantly higher EFI values than those harvested in the fall (Table 3).

The follicular epithelium in juvenile whales, irrespective of harvest season, had tall cuboidal to columnar epithelial cells lining the follicles (Table 3). In the fall, there was a significant difference in EFI values between juvenile and subadult or adult age classes.

There was no correlation between serum TH concentration and any of the serum, blubber, and liver OC metabolites and PCB congeners investigated (all  $P$  values  $> 0.05$ ).

## Discussion

Serum TH concentrations in bowhead whales were consis-

<sup>4</sup>Supplementary material for this article is available on the journal Web site (cjz.nrc.ca) or may be purchased from the Depository of Unpublished Data, Document Delivery, CISTI, National Research Council Canada, Building M-55, 1200 Montreal Road, Ottawa, ON K1A 0R6, Canada. DUD 5161. For more information on obtaining material refer to cisti-icist.nrc-cnrc.gc.ca/irm/unpub\_e.shtml.

**Table 1.** Thyroid hormone (total thyroxine (tT4), total triiodothyronine (tT3), free thyroxine (fT4), and free triiodothyronine (fT3)) concentrations in the bowhead whales (*Balaena mysticetus*) sampled in the BCBS stock (excluding pregnant females) and pregnant females only.

Whales sampled	Hormone	<i>n</i>	Mean	SD	Range
All (excluding pregnant females)	tT4 (nmol/L)	58	83.30	20.5	40.0–128.0
	tT3 (nmol/L)	56	1.14	0.42	0.10–2.20
	fT4 (pmol/L)	58	25.80	7.63	12.0–50.0
	fT3 (pmol/L)	54	4.22	1.63	1.50–10.40
Pregnant females only	tT4 (nmol/L)	5	59.00*	23.00	38.0–96.0
	tT3 (nmol/L)	5	1.04	0.29	0.80–1.50
	fT4 (pmol/L)	5	16.80*	6.34	9.00–23.0
	fT3 (pmol/L)	5	3.18	0.57	2.40–4.0

\*Significant difference from nonpregnant adult females at  $P < 0.05$ .

**Table 2.** Thyroid hormone (total thyroxine (tT4), total triiodothyronine (tT3), free thyroxine (fT4), and free triiodothyronine (fT3)) concentrations by age group and season (pregnant females excluded) in bowhead whales.

Hormone	Age group	Spring			Fall		
		<i>n</i>	Mean	SD	<i>n</i>	Mean	SD
tT4 (nmol/L)	Juvenile	10	79.6	21.6	19	86.4	19.0
	Subadult	3	92.0	26.5	12	75.0	23.2
	Adult	6	85.7	20.7	8	88.1	22.7
tT3 (nmol/L)	Juvenile	10	1.22	0.37	17	1.08	0.33
	Subadult	3	1.00	0.20	12	1.03	0.46
	Adult	6	1.08	0.28	8	1.45	0.63
fT4 (pmol/L)	Juvenile	10	29.4	7.63	19	26.6	5.23
	Subadult	3	27.7	2.89	12	21.8	7.57
	Adult	6	23.0	7.95	8	26.9	11.6
fT3 (pmol/L)	Juvenile	10	4.97	2.85	16	4.09	1.05
	Subadult	3	3.40	0.58	11	3.59	1.49
	Adult	6	4.20	0.99	8	4.73	1.08

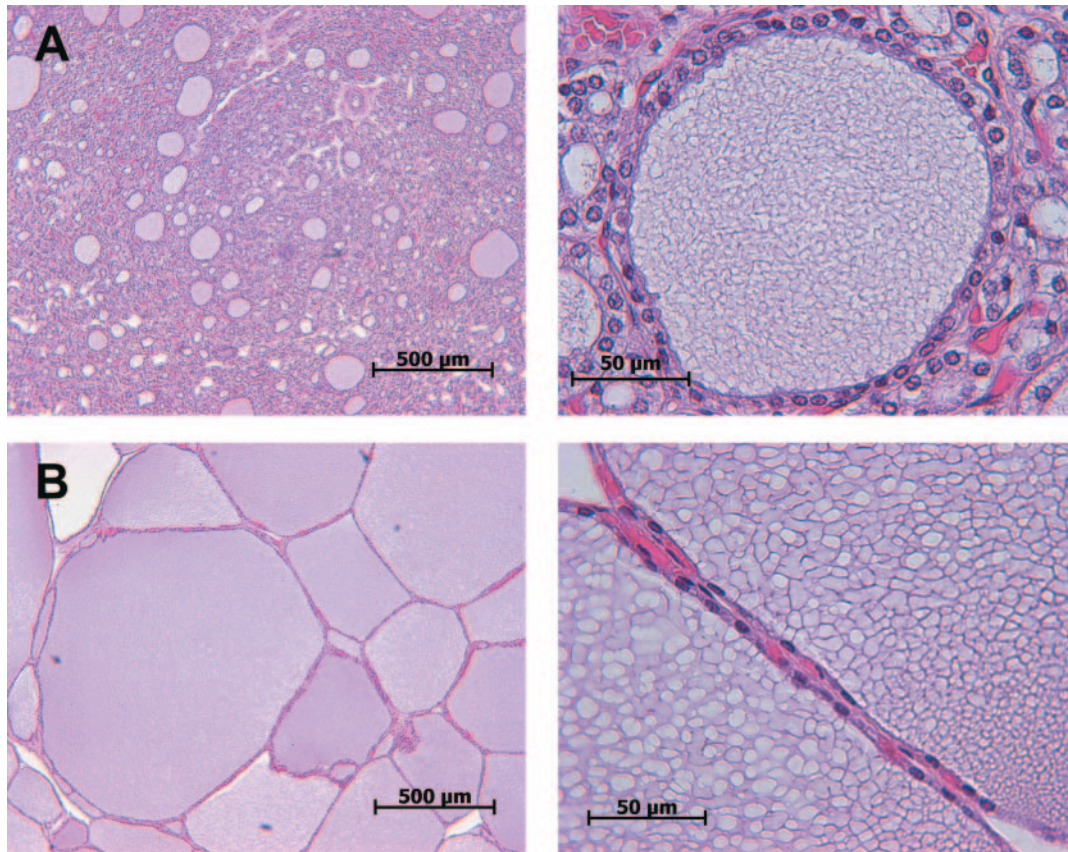
**Note:** There was no significant difference noted in any of the THs measured between seasons, sexes, or among age (length) groups.

tent among age classes (juvenile, subadult, and adult) and sex and reproductive groups, with pregnant females being the exception. The significantly lower serum TH concentration found in pregnant or lactating females is of interest, especially with respect to biomarker and health assessment interpretation. There is considerable variability in TH levels among mammalian species during pregnancy (Calvo et al. 1990; Kimura et al. 1990; Tomasi et al. 1998; Fantz et al. 1999). The fetal brain is known to be highly sensitive to deficient or excessive TH levels (Porterfield 1994). The iodine-rich diet of the bowhead whale is a likely determinant of the differences we observed in pregnant females, with the reduced maternal levels possibly serving to protect the fetus from high circulating iodine levels. In this study, the pregnant or lactating reproductive group consisted of five females; thus, it would be best to reevaluate these findings with a larger number of samples representing additional stages of pregnancy. The sample from the single fetus indicates that near-term fetal TH levels were high compared with the means for non-neonates, which is similar to ruminants and horses, where circulating concentrations of TH are much higher in neonates than in adults (Irvine and Evans 1975; Refsal et al. 1984).

No seasonal differences (fall versus spring) were found in

the serum TH levels in landed bowhead whales; however, we observed marked seasonal differences in the thyroid histology. Beluga whales exhibited seasonal changes in TH (St. Aubin and Geraci 1989). St. Aubin and Geraci (1989) suggested that this observed seasonal correlation relates to increased water temperature of the (inland) estuaries that belugas seasonally occupy. However, no such effect of water temperature was observed in studies of captive bottlenose dolphins (St. Aubin et al. 1996), indicating that these changes could be species-specific. TH concentration and histology were also examined in the beluga whale during different phases of their migration. Higher levels of circulating T3 and T4 were found in the summer months. This difference was accompanied by histological transformation of the thyroid follicular lining cells from a cuboidal to a columnar morphology (St. Aubin and Geraci 1989). In photoperiod studies of non-cetacean mammals, light restriction has been shown to increase the activity of the pineal gland, which results in increased melatonin production (Haldar et al. 1992; Bhagavan 2002). The neurohormonal properties of melatonin may affect the pituitary, hypothalamus, or metabolism of TH directly in laboratory animals. Melatonin has been reported to decrease blood T4 levels in both rats and hamsters (Singh et al. 1969; Vriend 1983; Haldar et al. 1992; Lewin-

**Fig. 1.** Histological appearance of the thyroid gland in the bowhead whale (*Balaena mysticetus*) by season. (A) Spring histological appearance of thyroid follicles. Note the numerous, small, and active follicles that are present in the gland, which is lined with cuboidal epithelial cells (top right panel). (B) Fall histological appearance of thyroid follicles. Note the large and distended follicles that are lined with flat and attenuated epithelium (bottom right panel).



**Table 3.** Measurement data of the epithelial-follicular index (EFI) in bowhead whales.

Whales sampled	EFI	SD	<i>n</i>	Season	<i>P</i>
All age classes	6.43	1.88	8	Spring	0.665*
	5.29	0.92	16	Fall	
Subadults and adults only	6.47	1.05	5	Spring	0.0016†
	4.10	0.74	9	Fall	
Juveniles only	6.35	0.58	3	Spring	0.655‡
	6.82	1.66	7	Fall	

\*No significant difference was found between spring and fall seasons.

†A significant difference was found between spring and fall seasons.

‡No significant difference was found between spring and fall seasons.

ski and Karbownik 2002). However, harp seal (*Pagophilus groenlandicus* (Erxleben, 1777)) studies did not indicate a stimulatory action of melatonin in the peripheral conversion of T4 to T3 (Stokkan et al. 1995). The effect of shortened day length on the histology of the thyroid in bowhead whales cannot be discounted, as the cell and glandular morphology suggest that the thyroid gland is substantially less active in fall than in spring. Assessment of photoperiod, including the measurement of serum melatonin, should be considered in future studies.

Intake of iodine from seasonal foraging may be an important factor in triggering the seasonal changes observed in follicular diameter and in the height of epithelial lining

cells. In the spring, bowhead whales are thought to be at the end of a period of winter fasting (Lowry 1993). Their prey is iodine rich (Krinsky 1965; Kon and Thompson 1949) and is seasonally available in large amounts (summer and fall) (Burns et al. 1993). Whales that migrate from the Bering Sea region to the Beaufort Sea region in spring are thought to have been fasting over winter, with intermittent feeding during spring migration as the productivity of the area increases over the summer months (Burns et al. 1993). In the normal mammalian thyroid gland, the limiting step of TH synthesis is the uptake of iodide (Bhagavan 2002; Pineda and Dooley 2003). Iodine levels are high in the blubber of marine mammals compared with adipose tissue

of terrestrial mammals (Ackman et al. 1975), making this tissue an important storage depot for this element. The colloid-dilated follicles noted in the fall indicate that storage is underway. The large, intensive intake of iodine during summer and fall feeding could lead to an adaptive decrease in TH synthesis known as the Wolff–Chaikoff effect (Wolff and Chaikoff 1948; Wolff 1969). This phenomenon involves the blockade of iodide organification and TH synthesis triggered by high intra-thyroidal iodide, which could potentially lead to changes in the histological appearance of the gland. In this scenario, during mid-summer and early fall, the thyroid initially becomes more active under the influence of increased dietary iodine (a period absent from our sampling) and other nutrients. When iodine levels reach a threshold of excess, this block occurs, effectively stopping the hydrolysis of T3 and T4 from thyroglobulin, resulting in colloid-dilated, inactive follicles that are characteristic of the histology noted in the fall for subadult and adult bowheads. Humans and laboratory rodents subsequently experience what has been termed an escape phenomena. This phenomenon allows serum TH levels to return to normal owing to inhibition of the symporter that introduces iodine into the cell. This causes a decrease in intracellular iodine, consequently removing the block in thyroid peroxidase expression which eventually leads to an increase in T4 and T3, thus sparing the organism from hypothyroidism in the presence of chronically increased levels of iodine (Wolff and Chaikoff 1948; Wolff 1969); this is a potential explanation for the normal serum TH concentrations noted in the presence of inactive thyroid histology and decreased EFI values in fall.

Juvenile whales maintained an active thyroid histologic appearance in both spring and fall. A relationship may exist between the persistence of this active epithelium and the rapid growth phase experienced during this phase of life (although serum TH levels do not reflect higher circulating TH levels). It is possible that this age class may convert, bind, or excrete TH in a different manner than subadult and adult whales. This difference could also be related to less efficient feeding mechanisms in these juveniles owing to shorter baleen and inexperience in feeding methods and sites. In this case, iodine exposure may be inadequate to induce the Wolff–Chaikoff block and escape, allowing for maintenance of a histologically active thyroid. Measurements of circulating and stored iodine levels are needed to further investigate these differences.

Published research provides compelling evidence that some OCs, including several parent PCBs and metabolites, alter the thyroid axis. Alterations in thyroid function and morphology have been induced by PCBs in the laboratory and in marine mammals (Bryne et al. 1987; Brouwer et al. 1989; Skaare et al. 2001; Debier et al. 2005), and have been related to the interactions of OCs with TH transport proteins. Hydroxylated OCs and other halogenated phenolic compounds are selectively retained in blood because of their structural similarities to T4 and binding affinity with thyretin (owing to similar chemical configuration at the OH binding site and surrounding halogen substitution at the vicinal C atoms) (Ishihara et al. 2003). TTR functions in the transport of both retinol and TH and accounts for ~20% of circulating T4 in experimental animals. Many OCs are simi-

lar in structure to TH and retinol. Often the affinity of the transport protein for these contaminants is far greater than for TH (Arctic Monitoring and Assessment Program (AMAP) 1998, 2002; Ishihara et al. 2003). Bowhead whale OC concentrations in serum, blubber, and liver were low compared with those in other Arctic marine mammals and were of a magnitude lower than those seen in large mysticete whales in the northern Atlantic Ocean (Hoekstra et al. 2002). This is likely attributable to the low trophic level on which the bowhead whale feeds and differences in OC deposition between eastern and western Arctic regions (Wagemann et al. 1990, 1996; Hoekstra et al. 2002, 2003). Measurement of TTR concentrations would be useful in making a more complete evaluation of TH binding dynamics in bowhead whales. It is possible that OC concentrations of the magnitude noted in this study are not sufficient to produce a measurable effect on TH or retinol. This may support the use of TH as a biomarker, as subclinical or clinical effects of these contaminants have not been seen in this stock. However, it is also possible that circulating TH levels in bowhead whales may not be an appropriate biomarker for low-dose exposure to OCs. In cases such as this, other biomarkers may be more appropriate. For example, hepatic type I monodeiodinase (MDI) is an enzyme involved in TH homeostasis. Research in other species has shown reduction in MDI activity to be a more sensitive indicator of PCB exposure (Gould et al. 1999). Hepatic type I MDI may have potential as an alternative biomarker in cases of low-dose exposure to OCs, although it has yet to be investigated in marine mammals. Cytochrome P450 monooxygenase 1A1 (CYP1A1) is another possible low-dose organochlorine exposure biomarker that has shown promise in other mammalian species, including several species of marine mammal and river otters (*Lontra canadensis* (Schreber, 1777)) (Fossi et al. 1997; Wolkers et al. 1999; Ben-David et al. 2001; Angell et al. 2004). Exposure to contaminants often results in increased CYP enzyme activities, but the CYP enzyme profiles are species specific; therefore, additional knowledge about CYP enzymes in the bowhead whale would be needed prior to being useful as a biomarker (Okey 1990; Wolkers et al. 1999).

The above data indicate some of the confounding variables encountered when using serum TH concentrations and thyroid histology as biomarkers. There are also issues of validity and reliability of measurements that need to be considered, as well as the capacity to interpret “impact” without assay validation and a sense of “normal”.

A degree of agreement must exist between the biomarker and the process it is measuring for it to be valid. In this case, we have low concentrations of OCs of interest measured (compared with other related mysticetes and marine mammals of the region) and a population experiencing positive growth that possess an arguably surprising lack of pathology (gross or histological) (George et al. 2004; C. Rosa, unpublished data). A lack of controlled experimental trials hinders full understanding of the response of TH to stressors in marine mammals, but this alone should not disqualify its utility as a biomarker. Most information on TH in marine mammals has been gathered retrospectively from wild populations, and data from rodent models are supportive of the theory of TH suppression in cases of exposure to certain lev-

els of OCs. Biomarker levels may undergo modifications in relation to the hormonal status, age, and sex of an organism; this limit can be minimized if the reproductive cycle, physiology, and variations between individuals of the species in question are known. Our results have found levels of serum TH to be remarkably stable among age classes, seasons, and between sexes. Age and seasonally related changes were noted in the histology and EFI values, but a robust control of serum TH levels appears to occur in the presence of these obvious changes. This may be attributable to homeostatic mechanisms that maintain serum TH levels in a normal range when intake of food (iodine) is marginal or excessive and may indicate that deviation from the norm is unlikely in apparently “healthy” individuals with low OC burdens. This lends greater credibility to the hypothesis that normal TH levels are maintained in this state and deviations from the norm are likely to be caused by something other than differences seen among age classes, seasons, and between sexes.

The accuracy of a given biomarker needs to be determined and reproducibility is important for it to be considered reliable. In fieldwork, this is difficult to realize. The subjects are wild, and their health and the contaminants that they are exposed to cannot be controlled. Even in captive marine mammals, subject numbers are low and the lack of ethical acceptance for clinical toxicological trials hinders full understanding of the behavior of many substances of interest. Multiple measurements of a biomarker from several animals with similar levels of a given contaminant are likely to differ. We have attempted to minimize this concern by gathering a large sample size of animals of both sexes, in two seasons, and among different age groups. However, questions remain as to how specific the effects of OCs are on TH concentrations and with what amount of accuracy serum TH represents the total body burden of OCs. This study is an example of more complete types of data collected alongside the limitation of dealing with larger cetacean species not amenable to captive research.

Previous TH investigations in marine mammals have produced variable results. Putatively, no correlation was seen between the low concentrations of OC congeners and serum TH levels in this healthy population of whales, and little variability in serum TH concentrations was detected among age, season, and sex groups (except in pregnant females). Consideration was given to previous studies that noted changes in TH levels with capture stress and captivity (St. Aubin and Geraci 1988). In the present data set, there was no correlation between the “time to death” and TH concentrations (C. Rosa, unpublished data), and indeed, in most cases, these times were quite rapid. These findings further support the use of TH concentration as a potentially reliable marker of OC exposure. However, the dynamics of TH production in fasting-adapted cetaceans warrant continued research. At present, we recommend the use of TH as a biomarker in conjunction with other endpoints that provide insight into the health and contaminant exposure of the individual. Results should be interpreted with caution. These data provide valuable information from a rare opportunity to collect samples from a large number of whales that are in good health, sampled across these strata. Future work involving cetacean TH and histology should be able to build on this study to formulate new hypotheses regarding TH

concentrations in this population and populations of other large mysticetes. It is important for additional data to be gathered, as this will add to our knowledge of TH dynamics and to the value of these hormones as biomarkers, not only of contaminants, but of ongoing (offshore industrial activities) and emerging (climate change) potential stressors.

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