Ultrasoundography is an effective imaging tool for assessment of thyroid glands and screening for pathological changes in this tissue in humans. Knowledge of normal ultrasonographic features is essential for diagnostic and treatment monitoring purposes. Investigators in previous studies have evaluated thyroid glands in humans via real-time ultrasonography, and thyroid glands in healthy people were described as having an echotexture of medium-to-high homogeneity.

Objective—To evaluate the use of ultrasonography for thyroid gland assessment in healthy Indo-Pacific bottlenose dolphins (Tursiops aduncus), describe the ultrasonographic appearance of the thyroid gland and adjacent anatomic structures, and identify potential associations between variations in thyroid gland morphology and demographic features in this species.

Animals—18 captive Indo-Pacific bottlenose dolphins.

Procedures—1,404 ultrasonographic examinations of the thyroid gland and adjacent anatomic structures (eg, cervical lymph nodes, musculature, and vasculature) were performed during the >3-year study period. Shape, echogenicity, and homogeneity of thyroid glands were assessed, and glands were categorized into morphological configurations on the basis of results of 2-D and 3-D ultrasonographic evaluation. Associations between demographic factors and thyroid gland morphology were assessed.

Results—Thyroid lobes appeared elliptical or fusiform in the transverse scan plane and round to oval in longitudinal scan planes; morphologically, glands comprised 2 lobes joined by an isthmus or a roughly diamond-shaped structure located on the ventral surface of the trachea. Major blood vessels and cervical lymph nodes were identified. Thyroid parenchyma was typically uniform and homogeneous, with echogenic reticulations and well-defined borders. Thyroid glands were hypoechoic or isoechoic relative to the sternocephalicus muscle; echogenicity was greater in adolescents than in adults. Thyroid gland volume differed between sexes, between sexually mature and immature dolphins, and among age groups and was positively correlated with body length and weight.

Conclusions and Clinical Relevance—Ultrasoundography provided a reliable and repeatable method for evaluation of thyroid glands and adjacent anatomic structures in live dolphins.
tures of thyroid glands in dogs, cats, and horses. Assessment of thyroid gland morphology and function is one of the outstanding diagnostic challenges in cetacean clinical endocrinology.3,24

Ultrasonographic measurement of thyroid glands in bottlenose dolphins has been reported, and efforts have been made to establish baseline values for variables related to thyroid function.6,7 However, these measurements were found to be extremely variable because of skill differences between operators and did not include any detailed information on adjacent structures and related anatomic landmarks in the region of the thyroid gland. Published information on the ultrasonographic evaluation of normal thyroid glands and the adjacent anatomic structures of marine mammal species, which may be crucial for the diagnosis of disease, is limited.25,26, 6

Factors such as demographic variables, physiologic cycles, and health status23,24,27–32 affect mammalian thyroid function and morphology and may potentially cause misdiagnosis of thyroid abnormalities. Studies23,31,33,34 of circulating hormone concentrations in cetaceans and pinnipeds have identified age-related changes in thyroid function and morphology. As these animals age (from postnatal development to maturation), thyroid function typically decreases. Thyroid hormones are also essential for growth and organ development,35,26 and overt hypothyroidism and hyperthyroidism may be associated with body weight changes in many species.37–39 The authors of 1 study23 reported a decrease in circulating free thyroxine concentrations with the onset of maturity in a population of wild dolphins.

Ultrasonography was suggested to be more sensitive than serum thyroid-stimulating hormone measurements for the assessment of thyroid abnormalities in humans.40 To the authors’ knowledge, the literature is devoid of any reference to possible influences of variables such as age, sex, sexual maturity, body length, and weight on thyroid morphology in bottlenose dolphins. The purpose of the study reported here was to evaluate the use of ultrasonography for thyroid gland assessment in healthy Indo-Pacific bottlenose dolphins and to describe the ultrasonographic appearance of the thyroid gland and adjacent anatomic structures in this species. We also sought to identify potential associations between variations in thyroid morphology and various demographic factors.

Materials and Methods

Animals and management—Eighteen (7 male and 11 female) Indo-Pacific bottlenose dolphins (Tursiops aduncus) at Ocean Park, Hong Kong, China, were included in the study. Diets consisted of various proportions of capelin, sardine, herring, and squid, with vitamin and mineral supplements. From August 1, 2006, to January 30, 2009, ultrasonographic evaluations of each dolphin were performed every week, for a total of 1,404 evaluations. All dolphins involved in the study were trained to cooperate for ultrasonographic examinations. The animals were apparently healthy, with no recent history of illnesses, and were not receiving medication expected to alter thyroid gland physiology during the study. Serum concentrations of free and total thyroxine and triiodothyronine were also determined at least once during the study for each dolphin, and the values were all within established reference ranges.24 The study was licensed under the Animals Control of Experiments Ordinance, Cap 340, issued by the Department of Health of Hong Kong Special Administrative Region. All procedures were reviewed and approved by the Animal Subjects Ethics Sub-committee of the Hong Kong Polytechnic University and the Scientific Advisory Committee of Ocean Park Hong Kong.

Length and weight evaluations—From the beginning of the study, body weight and length measurements of each dolphin were conducted monthly, during the first feeding in the morning. Body length measurements were obtained with the dolphin lying flat and straight at the bottom of the pool, in the shallow water against the wall of the poolside. Two targets, 1 at the tip of the rostrum and 1 at the notch between the flukes, were placed by 2 trainers to ensure that the dolphin was kept in a straight posture. A flexible measuring tape was placed along the 2 targets. Body weight measurements were obtained with the dolphin coaxed to slide out of the water and lie down on a calibrated scale platform.

Determination of age and age groups—Age assessment of 8 dolphins that had been collected from the wild was performed by a resident veterinarian (REK) at the facility on the basis of their body length, weight, growth patterns, and length of time in captivity. Ages were known for the remaining 10 dolphins, which were born in captivity. On the basis of age at the time of ultrasonographic examinations, collected data were categorized into 3 groups: ≤ 5 years old (calf), > 5 to 10 years old (adolescent), or > 10 years old (adult). At the end of the study, 4 dolphins were calves, 5 were adolescents, and 9 were adults; mean age of the population was 16 years (median, 11.5 years; range, 1 to 37 years); mean body weight was 129.2 kg (median, 128.5 kg; range, 98.0 to 185.1 kg); and mean body length was 218.0 cm (median, 218 cm; range, 196 to 244 cm).

Assessment of sexual maturity—Ultrasonographic evaluation of the ovaries was performed for all female dolphins by veterinarians at the facility who had experience in performing this procedure. Archived ultrasonographic images and examination reports were further evaluated by an experienced ultrasonographer (FMB). Both ovaries were assessed in the examinations, and the ovarian cortex was assessed during different stages of the reproductive cycle. For purposes of a concurrently performed study41 of male dolphin reproductive status, weekly semen collections were conducted from the beginning of the study. Sexual maturation of female dolphins was determined as the date of first ovarian activity (evidenced by follicle maturation), whereas sexual maturation of male dolphins was determined as the date of first detection of spermatozoa in the ejaculate. At the end of the study, 14 dolphins were sexually mature and 4 were sexually immature. Two animals that became sexually mature during the study period had measurements recorded for both categories.

Ultrasonographic examination of the thyroid gland—All ultrasonographic examinations were per-
formed by an imaging specialist (BCWK) with a fully equipped clinical ultrasound unit in conjunction with a 2- to 6-MHz curvilinear 3-D broadband curved array transducer and a 2- to 5-MHz 2-D broadband curved array transducer or with a portable ultrasound unit in conjunction with a 2- to 5-MHz curvilinear transducer. Low frequency curvilinear transducers were used, which provided an appropriate depth of images and included the thyroid gland and adjacent structures in a single image scan plane. All images were recorded by use of direct digital capture or a thermal printer. Because there was no layer of air between the skin surface and the transducer, no coupling gel was required.

The dolphins were trained to approach the poolside and position themselves in dorsal recumbency, with the fluke supported by a trainer. The transducer was introduced onto the thorax region and slowly moved cranially past the sternum.

For 2-D ultrasonography, the transducer was placed in a transverse orientation at the thoracic inlet, midway between the insertions of the pectoral fins. The transducer was moved cranially until a brachiocephalic vein was identified (Figure 1). The transducer was then advanced cranially until the transverse dimensions of the left and right lobes of the thyroid gland were identified. The transducer was then rotated 90° to visualize the longitudinal dimensions of different portions of thyroid gland. The transducer was also placed in an oblique manner to visualize the long axis of the thyroid lobes and produce measurements for thyroid volume calculations.

For 3-D ultrasonography, the thyroid gland was initially identified via 2-D ultrasonography, and then the 3-D data acquisition function was activated. The thyroid gland was scanned in a single sweep with a sweeping angle of 50°, which was adequate to encompass the entire thyroid gland. The 3-D images were reconstructed automatically by a built-in specific quantification software program. The software program allowed manipulation of reconstructed slices in 3 image scan planes (transverse, reconstructed longitudinal, and reconstructed coronal) of the thyroid gland. The 3-D images were stored on the hard drive of the ultrasound unit. After scanning, the set of 3-D images was retrieved and thyroid configuration was determined with the software, which assessed the different levels of coronal scan planes. Thyroid glands of all dolphins were categorized into 1 of 4 gross configurations via 2-D and 3-D ultrasonographic evaluation in the described scan planes as follows: type A = 2 lobes (1 on each side of the trachea) joined by an isthmus; type B = 2 separate lobes (usually of equal size; 1 on each side of the trachea) with no connecting isthmus; type C = a shield-like, roughly diamond-shaped single structure located on the ventral surface of the trachea; and type D = an irregular, multilobular (grape cluster-like) structure, with adjacent but separate lobules.

Shape, degree of border definition, echogenicity, and homogeneity of the thyroid gland were evaluated. The shape of each thyroid lobe was subjectively described as fusiform or elliptical in the transverse scan plane and irregular or round to oval in the longitudinal scan plane. Border definition was assessed according to the smoothness of the margin between the thyroid gland and surrounding soft tissues as well-defined or ill-defined. Echogenicity of the thyroid gland was subjectively compared with that of the sternocephalicus muscle in the same image and classified as one of

![Figure 1](image1.jpg)

Figure 1—Representative transverse ultrasonographic image of the neck of an Indo-Pacific bottlenose dolphin (*Tursiops aduncus*) in a study to evaluate ultrasonographic features of the thyroid gland and adjacent structures in 18 dolphins of various ages and sexes. Notice the left brachiophecalic vein (white arrows), left and right brachiophecalic trunks, and omooccipital artery (black arrows). LBT = Left brachiophecalic trunk. OA = Omooccipital artery. RBT = Right brachiophecalic trunk.

![Figure 2](image2.jpg)

Figure 2—Images depicting ultrasonographic measurement of the maximum transverse dimension of the thyroid gland in bottlenose dolphins. A—Photograph showing the position of the transducer used to obtain transverse scan plane images at the ventral aspect of the neck of a dolphin trained to cooperate for ultrasonographic examinations. B—Schematic diagram of the thyroid gland (coronal orientation) with the dashed line representing the position of the transducer. C—Transverse grayscale ultrasonographic image of the thyroid gland. Notice the markers indicating the maximum transverse dimension of the thyroid gland (+).
the following: hypoechoic, isoechoic, or hyperechoic. Echogenicity of the left and right thyroid lobes was subjectively compared with the isthmus in the same longitudinal image. The thyroid parenchyma was categorized as homogeneous or heterogeneous on the basis of the presence or absence of any nodules or space-occupying lesions.

To obtain thyroid gland measurements, the transducer was moved cranially and caudally until the scan plane showing the maximum transverse dimension of the thyroid gland was found and measured (Figure 2). The transducer was then rotated 90° to obtain longitudinal scan plane images of the thyroid gland. A full survey of the thyroid gland was performed in the longitudinal planes with the transducer moved from the left to the right. Images of the following 3 longitudinal scan planes were recorded: midline of the thyroid, maximum longitudinal dimension of the left lobe, and maximum longitudinal dimension of the right lobe (Figures 3 and 4). In each longitudinal scan plane, the ventrodorsal dimension and the craniocaudal dimension of the evaluated thyroid region were measured. Thyroid volume was determined by use of the following ellipsoid equation:

$$\text{Thyroid volume} = \frac{\pi}{6} \times TS_{\text{MAX}} \times \text{Sum}_{\text{vd}} \times \text{Sum}_{\text{cc}}$$

where $TS_{\text{MAX}}$ is the maximum transverse dimension (ie, width) of the thyroid gland, $\text{Sum}_{\text{vd}}$ is the mean value of the ventrodorsal dimension (ie, thickness) mea-

Figure 3—Images depicting ultrasonographic measurement of the longitudinal dimension of the thyroid gland along the midline in a bottlenose dolphin. A—Photograph showing the position of the transducer used to obtain longitudinal scan plane images at the ventral aspect of the neck. B—Schematic diagram of the thyroid gland (coronal orientation) with the dashed line representing the position of the transducer. C—Longitudinal ultrasonographic image of the thyroid gland (diagonal lines). Notice the markers indicating the ventrodorsal (x) and craniocaudal (+) dimensions.

Figure 4—Images depicting ultrasonographic measurement of the maximum longitudinal dimensions of the left (A through C) and right (D through F) lobes of the thyroid gland in a bottlenose dolphin. A—Photograph showing the position of the transducer used to obtain longitudinal scan plane images of the left lobe of the thyroid gland. B—Schematic diagram of the thyroid gland (coronal orientation). The dashed line represents the position of the transducer over the left lobe. C—Longitudinal ultrasonographic image of the left lobe of the thyroid gland. D—Photograph showing the position of the transducer used to obtain longitudinal scan plane images of the right lobe of the thyroid gland. E—Schematic diagram of the thyroid gland (coronal orientation) with the dashed line representing the position of the transducer over the right lobe. F—Longitudinal ultrasonographic image of the right lobe of the thyroid gland (diagonal lines). The maximum longitudinal dimension of each thyroid lobe is shown. Notice the markers indicating the ventrodorsal (x) and craniocaudal (+) dimensions of thyroid lobes in ultrasonographic images.
Ultrasonographic examination of structures in the neck adjacent to the thyroid gland—Structures adjacent to the thyroid gland, such as cervical lymph nodes, musculature, and vasculature, were identified, and images were obtained. The transducer was placed at the thoracic inlet, located transversely between the insertion of the sternum and cranial poles of the thyroid lobes and longitudinally between the insertion of the pectoral fins, and various scan planes and angles were used for visualization of these structures.

Statistical analysis—Three-factorial ANOVA was conducted to determine significant differences in thyroid volume among dolphins grouped according to demographic factors such as age, sex, and sexual maturity. For the analysis, thyroid volumes were determined after a log-logit transformation of the standard curve to meet assumptions of normality and equal variance, and values were expressed as mean ± SEM. Pearson product-moment correlation was determined to investigate associations between thyroid volume and body weight or body length. Differences in thyroid gland homogeneity, echogenicity, and border definition among dolphins grouped according to age, sex, and sexual maturity were assessed separately with Fisher exact tests. For all statistical tests, values of \( P < 0.05 \) were considered significant.

Results

Ultrasoundographic appearance of the thyroid gland—In total, 1,404 evaluations of the thyroid glands of 18 dolphins were performed during the study; 1,384 were included in analysis of associations between measurements and various demographic factors. The number of evaluations performed varied among dolphins (range, 10 to 72). Two dolphins died during the study period. One of the 2 deceased dolphins had a diagnosis of severe acute cardiomypathy, and the other had viral infection of the liver leading to acute hepatic necrosis; these animals had 10 and 44 evaluations performed, respectively. Results of histologic analysis revealed that the thyroid glands of these dolphins appeared to have been active and to have normal cellular architecture. The thyroid glands of all dolphins were clearly visualized in all ultrasonographic images. The time to conduct a complete ultrasonographic examination of the thyroid gland was approximately 3 to 5 min/dolphin.

The shape of the thyroid lobes appeared ellipsoidal or fusiform in the transverse scan plane and round to oval in the longitudinal scan plane (Figure 5). The thyroid capsule was usually echogenic, and the borders of the thyroid gland were well-defined and smooth in 15 of 18 dolphins, whereas ill-defined borders were observed in 3 animals (from the time of initial evaluation [3, 26, and 37 years of age]). There was no significant effect of age, sex, or sexual maturity on thyroid gland border definition.

The echopattern of thyroid parenchyma was uniform and homogeneous in 14 of 18 dolphins, consisting of a dense agglomerate of very fine small echoes of approximately equal size, with the presence of echogenic reticulations (Figure 5). In the remaining 4 dolphins, the thyroid gland had a heterogeneous distribution of echoes with hyperechoic foci, isoechoic foci, hypoechoic foci, or a mottled appearance. There was no significant effect of age, sex, or sexual maturity on thyroid gland homogeneity. Intrathyroidal vessels appeared as small rounded structures on images of scan planes perpendicular to the axis of the vessel or as small linear structures on images of scan planes parallel to the axis of the vessel.

The thyroid gland was typically hypoechoic (4 of 5 calves, 1 of 6 adolescents, and 8 of 9 adults) or isoechoic (1 calf, 5 adolescents, and 1 adult), compared with the adjacent sternoecephalic muscle, and was not significantly different among age groups. Two dolphins changed age groups during the study (1 from calf to adolescent and 1 from adolescent to adult) and were included in both. Thyroid echogenicity was significantly \((P < 0.05)\) different between adolescents and adults; however, sex did not have a significant effect on this variable. Echogenicity of the right and left thyroid lobes was significantly \((P < 0.05)\) different (either right or left lobe, hyperechoic to the isthmus) from that of the isthmus in 12 of 18 dolphins.

The thyroid glands of all 18 dolphins were categorized on the basis of gross configuration as either type A (2 lobes [1 on each side of the trachea] joined by an isthmus; \( n = 9 \)) or type C (a shield-like, roughly diamond-shaped single mass, located on the ventral surface of the trachea; 9). Accessory thyroid tissue (a pyramidal lobe) was detected in 7 dolphins and was visualized best in the longitudinal scan plane with a small dorsoventral diameter of 5 to 8 mm and craniocaudal diameter of 11 to 21 mm (Figure 5). Origins of the pyramidal lobes included the right thyroid lobe \(( n = 3 )\), isthmus \((3)\), and left thyroid lobe \((1)\).

Thyroid gland volume—Age, sex, sexual maturity, body length, and weight influenced thyroid gland volumes. The thyroid volume of calves \((15.65 ± 0.16 \text{ cm}^3; n = 268 \text{ evaluations})\) was significantly \((P < 0.05)\) and \((P < 0.001, \text{ respectively})\) greater than that of adolescents \((14.62 ± 0.24 \text{ cm}^3; 315)\) and adults \((10.65 ± 0.16 \text{ cm}^3; 801)\). Moreover, thyroid volume of adolescents was significantly \((P < 0.001)\) greater than that of adults, and that of females \((12.81 ± 0.17 \text{ cm}^3; n = 910)\) was significantly greater than that of males \((11.98 ± 0.18 \text{ cm}^3; 474)\).

Among adolescent and adult dolphins, there was a significant difference in thyroid volume between sexes; adolescent females had significantly \((P < 0.001)\) greater thyroid volumes \((16.61 ± 0.22 \text{ cm}^3; n = 220 \text{ evaluations})\) than did adolescent males \((10.00 ± 0.18 \text{ cm}^3; 95)\), and adult females had significantly \((P < 0.001)\) greater thyroid volumes \((11.03 ± 0.21 \text{ cm}^3; 315)\) than did adult males \((9.60 ± 0.22 \text{ cm}^3; 210)\). However, there was no significant effect of sex on thyroid volume in calves.

Thyroid volume of sexually immature dolphins \((15.60 ± 0.16 \text{ cm}^3; n = 239 \text{ evaluations})\) was significantly \((P < 0.001)\) greater than that of sexually mature dolphins \((11.92 ± 0.15 \text{ cm}^3; 1145)\). Sexually immature males had significantly \((P < 0.001)\) greater thyroid vol-
umes (16.05 ± 0.15 cm³; n = 169) than did sexually immature females (14.51 ± 0.40 cm³; 70). However, sexually mature males had significantly ($P < 0.001$) smaller thyroid volumes (9.72 ± 0.16 cm³; n = 305) than did sexually mature females (12.66 ± 0.18 cm³; 840).

Significant positive correlations of thyroid volume with body length (n = 280 comparisons; $r = 0.565; P < 0.001$) and body weight (311; $r = 0.407; P < 0.001$) were detected; variables were excluded if dolphins were uncooperative during measurement. The $r^2$ coefficients indicated that approximately 32% and 17% of the variance in thyroid volume could be predicted from body length and body weight, respectively.

Structures in the neck region adjacent to the thyroid gland—Ultrasoundographically, the skin and blubber measured in 18 dolphins corresponded to a thick hypoechoic band ranging from 14.8 to 20.3 mm in thickness. The pretracheal layer of the deep cervical fascia formed a detectable sheath encasing the sternocleidomastoid and sternohyoid muscles, which were easily visualized in ultrasonographic images. Although thickness of these muscles appeared to vary among individual dolphins, the sternocleidomastoid and sternohyoid muscles could be recognized as elongated structures at the ventral aspect of the thyroid gland. The sternocleidomastoid muscle appeared to be slightly hyperechoic or isoechoic to the thyroid parenchyma, whereas the 2 sternohyoid muscles were either isoechoic or hypoechoic, compared with the thyroid gland.

The dorsal surface of the thyroid gland was in contact with the ventral surface of the trachea. The trachea was identified ultrasonographically as a hyperechoic line with posterior acoustic shadowing (Figure 5).

The lateral surfaces of the thyroid gland included the major cervical vessels embedded within the carotid sheath and cricoid cartilage. Major cervical vessels comprised the internal and external carotid arteries (located immediately lateral to the thyroid lobes) and internal jugular vein (located lateral to the internal and external carotid arteries and spontaneously visible to some degree). Cricoid cartilage was identified as an oblique hyperechoic line lateral to the carotid arteries and the internal jugular vein in ultrasonographic images (Figure 5).
Arteries (eg, brachiocephalic trunk, subclavian arteries, internal and external carotid arteries, omocipital arteries, and superior thyroid arteries) and veins (eg, brachiocephalic veins, internal jugular veins, and superior thyroid veins) in the neck region were easily visualized. The superior thyroid arteries arose from the brachiocephalic trunk, supplying blood to the thyroid gland, and the superior thyroid veins drained into the internal jugular veins (Figure 6).

Cervical lymph nodes were also visualized via ultrasonography. The lymph nodes were isoechoic to hypoechoic, compared with the adjacent sternocleidomastoid muscle (asterisk), and are oval in shape with an echogenic hilus (arrowhead). Arteries (eg, brachiocephalic trunk, subclavian arteries, internal and external carotid arteries, omocipital arteries, and superior thyroid arteries) and veins (eg, brachiocephalic veins, internal jugular veins, and superior thyroid veins) in the neck region were easily visualized. The superior thyroid arteries arose from the brachiocephalic trunk, supplying blood to the thyroid gland, and the superior thyroid veins drained into the internal jugular veins (Figure 6).

Cervical lymph nodes were also visualized via ultrasonography. The lymph nodes were isoechoic to hypoechoic, compared with the adjacent sternocleidomastoid muscle, and round or oval in shape, with an echogenic hilus (Figure 7). Cervical lymph nodes were usually found between the sternocleidomastoid and sternohyoid muscles, cranial and lateral to the thyroid gland, and around the mediastinum and brachiocephalic veins, at a depth of 40 to 70 mm from the ventral surface.

Two to 3 lymph nodes (including both sides) were identified in each dolphin for a total of 44 lymph nodes in 18 animals. The mean long axis measurement of lymph nodes was 24 mm (median, 26 mm; range, 20 to 35 mm).

Discussion

Assessment of the thyroid gland and adjacent anatomic structures in dolphins has been performed in postmortem studies. The thyroid gland in these animals is not palpable during clinical examination because of the presence of a thick blubber layer and strong neck muscles. Although serum concentrations of thyroid hormones are useful for evaluation of thyroid function, blood sample monitoring may be performed infrequently because of the invasiveness of the procedure. To date, there is scant information available on noninvasive, real-time assessment of the morphology of the thyroid gland and adjacent structures in this species. In a previous study, the thyroid glands of 2 Atlantic bottlenose dolphins were assessed via ultrasonography; however, no details were given on the normal ultrasonographic appearances of the thyroid glands and adjacent structures in those animals. In the present study, we applied a simple scanning protocol with techniques that were useful for repeatable ultrasonographic thyroid gland examination. We also documented the ultrasonographic features of apparently normal thyroid glands and adjacent structures in a group of healthy captive Indo-Pacific bottlenose dolphins and identified variations of thyroid gland morphology that were associated with selected demographic factors.

The ultrasonographic appearance of dolphin thyroid glands in the present study corresponded well with the previously reported gross anatomy, and many features were similar in all of the dolphins. The borders of the thyroid gland were usually well-defined, which was not surprising because the thyroid gland in dolphins is encapsulated, providing a fairly uniform surface for ultrasound beam reflection. Previous reports in humans and harbor porpoises have indicated that increased age corresponds with a possible increase in adipose tissue deposition and connective tissue proliferation surrounding the thyroid gland, leading to a decrease in the acoustic impedance difference between the thyroid parenchyma and the adjacent soft tissues. Although no significant effect of age on thyroid border definition was detected in dolphins of the present study, similar changes may explain the ill-defined thyroid borders identified in 2 dolphins that were > 25 years old.

In an earlier study, evaluation of gross morphologic revealed that the thyroid gland in dolphins typically appeared to be compact and homogeneous beginning in infancy, which may account for the generally uniform and homogeneous echopattern of thyroid glands in ultrasonographic examinations in the present study. Additionally, the presence of echogenic reticulations within the thyroid gland may be due to the presence of fibrous bands, which are considered to be normal and to increase in number with advancing age. Although the variability of follicle size and colloid density have been reported to apparently increase with age, differences in homogeneity of the thyroid gland among...
the 3 age groups in the present study were not significant and the structure typically appeared homogeneous in ultrasonographic images. Various thyroid disorders in cetaceans have been identified and histologically described. Underlying thyroid disorders may explain the heterogeneous ultrasonographic appearance of the thyroid gland in 4 dolphins of the present study, although all dolphins had serum concentrations of free and total thyroxine and triiodothyronine within established reference ranges when evaluated during the study. Further studies are suggested to correlate histologic findings with ultrasonographic findings in dolphins with heterogeneous echopatterns in this tissue.

In the present study, relative echogenicity of the thyroid gland differed significantly \((P < 0.05)\) between adolescent and adult dolphins. The thyroid gland was typically hypoechoic, compared with the adjacent sternocleidomastoid muscle, in calves (4/5) and adults (8/9), whereas the thyroid gland was usually isoechoic in adolescents (3/6). Echogenicity of the thyroid gland has been reported to coincide with the architecture of the organ (follicular size and colloid density). Thus, changes in the histologic appearance may reflect changes in functional status as well as presence of thyroid abnormalities. Another study in cetaceans found that the thyroid gland of long-finned pilot whales had noticeable variation in size and appearance with increasing age, although active formation of follicles was reported in all stages of development in Pacific white-sided dolphins. Thyroid follicles have a fairly regular appearance in young animals; the colloid stains readily (slightly chromophilic) and has a fluid appearance, and the epithelial cells are tall. In older animals, the colloid is less hydrated in appearance and a large number of lamellar basophile masses are present in the thyroid gland. Investigators who studied the thyroid morphology of 60 stranded Atlantic bottlenose dolphins reported that in young dolphins, areas predominantly occupied by small follicles were scarcely recognizable as thyroid tissue, with the colloid-free follicles appearing as cell clusters rather than follicles.

In humans, the interface between thyroid cells and colloid has high acoustic impedance, causing more ultrasound waves to be reflected back to the transducer, giving a hyperechoic appearance to the thyroid gland relative to the sternocleidomastoid muscle. Results of previous studies indicated that thyroid cells in dolphins were smaller than those in humans, resulting in lower echogenicity. Therefore, in the thyroid gland of dolphin calves, the hyperechoic appearance relative to the sternocleidomastoid muscle may indicate small, actively growing follicles evidencing enhanced thyroid function or activity to fulfill higher metabolic needs for somatic growth. Thyroid development is followed by the maturation of larger follicles at the adolescent stage, and the thyroid gland in adolescent dolphins in the present study was typically isoechoic to the sternocleidomastoid muscle, likely due to the increased interface between thyroid cells and the high acoustic impedance of colloid. At the adult stage, the thyroid gland may develop architectural changes that are influenced by sex hormones as well as possibly develop signs of degeneration or subclinical thyroid abnormalities, and this is likely to contribute to a loss of echogenicity. Given that there were no clinical signs of thyroid function disorders observed in the investigated dolphin population, in our opinion, a dolphin thyroid gland that appears hypoechoic and isoechoic to the sternocleidomastoid muscle in ultrasonographic images may be considered normal.

In addition, variations in echogenicity between the left and right lobes or in different regions of a single thyroid gland could be possibly explained by uneven distribution of various sizes of follicles. Investigators in an earlier study reported that irregular or oval follicular lumens were seen in the parenchyma of the thyroid gland, with the size of the follicular lumen appearing larger in the central regions than in the peripheral regions in Risso dolphins.

In the present study, 2 of a possible 4 gross configurations of the thyroid gland were found in the studied population of dolphins. The first was type A, in which 2 thyroid gland lobes were connected by an isthmus, and the second was type C, in which there was a shield-like, diamond-shaped single mass located ventrally on the trachea. It is unknown whether the configuration is determined by local environmental influences or possible progressive changes throughout thyroid development or if this may be genetically influenced. A previous study in which these 4 configurations of dolphin thyroid glands were described used wild stranded animals for investigation, whereas in the present study, we evaluated a captive population of apparently healthy dolphins, with a mixture of captive-born and wild-caught animals living in captivity up to approximately 30 years. Longitudinal studies should be performed on the thyroid gland of captive-born dolphins to investigate the possible factors affecting the configurations.

Major blood vessels in the neck region adjacent to the thyroid gland of dolphins were visualized and identified via ultrasonography in the study reported here, and the description of these vessels has importance for future applications. Dolphins’ well-developed brains and associated specialized anatomic and physiologic adaptations to their aquatic environment have long attracted research interest. With the application of Doppler ultrasonography, hemodynamic studies of features such as vascular patterns, vascular resistance, and blood flow velocity of these major blood vessels in dolphins can be performed without use of invasive techniques such as angiography.

Cervical lymph nodes were also assessed via ultrasonography in the present study. The lymph nodes were isoechoic to hypoechoic, compared with the adjacent sternocleidomastoid muscle, primarily because of the presence of loosely organized lymphatic tissue. The lymph nodes were encapsulated by dense connective tissue with numerous elastic fibers that maintained their round or oval shape, as previously described. The hila of most of the lymph nodes were echogenic because of numerous interfaces from medullary cords and nodal vessels. These features all corresponded to those found in most terrestrial mammals. In a previous study, as many as 10 lymph nodes (typically, 6 to 8) were found at the aortic arch region and were always associated with the thymus and the thyroid gland. In the
present study, only 2 to 3 nodes were typically detected in each dolphin, possibly because of an echogenic similarity to surrounding fatty tissue.

In dolphins of the present study, thyroid volume significantly ($P < 0.001$ for all comparisons) decreased with increased age. From an energy use perspective, as body length and weight increase with increasing maturity, the surface area-to-volume ratio of an animal decreases, thereby reducing overall heat loss per unit of body weight. Consequently, a decrease in metabolism and thyroid volume may be observed.

Calves typically have a thinner layer of blubber with lower lipid content, compared with that of adolescents and adults, and this may not be adequate to provide sufficient insulation. Because their smaller body length and weight results in a larger surface area-to-volume ratio, dolphin calves may encounter greater heat loss or be more susceptible to cold temperatures than are adult dolphins. The thinner blubber of calves and higher metabolic rates to compensate for heat loss may account for their having the greatest thyroid volume among the 3 age groups in the present study.

Although not evaluated, the duration of captivity may also have influenced the variation in thyroid volume among different age groups of dolphins in the present study. A constant food supply and other conditions of captivity may result in degeneration of the thyroid gland with advancing age in captive dolphins, leading to the observed decrease in thyroid volume. Investigators of previous studies reported that the mean follicle diameter of the thyroid gland in captive dolphin populations appeared to be smaller than that of wild dolphin populations, with some captive dolphins having only remnants of the condensed and densely stained colloid, suggesting severe thyroid depletion. Thyroid depletion in adult dolphins may suggest iodine absorption impairment, dietary iodine deficiency, or increased utilization and turnover rate of iodine. Further studies are needed to investigate the possibility and implications of chronic thyroid depletion in aging captive dolphin populations.

Adolescent and adult females in the present study also had significantly ($P < 0.001$ for all comparisons) greater thyroid volume, compared with males of the same age groups; however, no significant difference in thyroid volume was found between male and female calves. At the adolescent and adult stages, thyroid growth may no longer be primarily influenced by factors involved with somatic growth but could be further modulated by sex hormones associated with reproductive development. Sex hormone steroid receptors have been identified in normal mammalian thyroid tissues and in those with pathological changes, and it has been reported that estrogen and androgen might be associated with promoting and inhibitory effects, respectively, on thyroid tissues. This may account for the significantly greater thyroid volume observed in female dolphins at the adolescent and adult stages.

Sexually immature dolphins had significantly ($P = 0.001$) greater thyroid volumes than did sexually mature dolphins. This may be attributable to a dominancy of somatic growth demands instead of reproductive development at this stage. Sexually immature dolphins would have higher metabolic demands for thermoregulation as well as organ growth and development and thus have a greater thyroid volume. The emphasis on somatic growth in sexually immature dolphins is expected to achieve a larger body size for optimal reproductive fitness because energy is invested only in the animal’s development, differentiation, and maintenance. When a dolphin reaches sexual maturity, thyroid physiology becomes further complicated by cyclic influences of sex hormones in females; in males, these influences are not as pronounced.

The present study revealed that sexually immature males had significantly ($P < 0.001$) greater thyroid volume than did sexually immature females, whereas sexually mature females had significantly ($P < 0.001$) greater thyroid volumes than did sexually mature males. Sexual bimaturism is a typical feature of marine mammals. As members of a polygynous species, male bottlenose dolphins attain sexual maturity later than do females. This allows additional time for somatic growth, giving males a larger body size before competing for females after reaching sexual maturity. Compared with sexually mature males, sexually mature females direct more of their energy expenditure on reproduction, particularly during pregnancy and lactation, resulting in increased thyroid volume to augment their metabolic processes. Further study could be useful to understand the underlying associations between thyroid morphology and reproductive events.

In dolphins of the present study, body length and body weight were positively correlated with thyroid volume but to different extents. Positive significant correlations between dolphin thyroid weight, standard body length, and body mass have previously been described. In the present study, body weight correlated weakly with thyroid volume, which may be attributable to a relative decrease in thickness of the blubber layer as the animal increases in size. It has been suggested that thyroid weight increases more slowly than does overall size in bottlenose dolphins. In the present study, body length was found to have a stronger correlation with thyroid volume than did weight, similar to previous findings in bottlenose dolphins. This is likely because body weight is more strongly influenced by health and nutritional status than is body length.

In the study reported here, ultrasonography provided a reliable and repeatable method for assessment of thyroid gland morphology in dolphins. With practice, locating the thyroid gland for routine clinical and follow-up examinations was quick and easy. The technique can also be applied to other cetacean species, including Pacific white-sided dolphins (Lagenorhynchus obliquidens) and beluga whales (Delphinapterus leucas). Established knowledge of the normal ultrasonographic anatomy of dolphin thyroid glands and adjacent structures as well as the identification of determinants of dolphin thyroid morphology will provide a normative reference to clinically recognize and treat thyroid gland abnormalities in living dolphins. This information would also be helpful for facilities in attaining self-sustainability and allowing optimal management of captive dolphins. Development of imaging techniques for consistent and reproducible monitoring of thyroid
glands in cetacean species during treatment for thyroid disorders could provide valuable insight to aid in understanding thyroid physiology and pathology in wild and captive cetaceans.


d. QLAB 5.0. Philips Medical System, Bothell, Wash.

e. Aloka Co Ltd, Mitakacho, Tokyo, Japan.

f. SPSS for Windows, version 16.0, SPSS Inc, Chicago, Ill.

g. GraphPad InStat, version 3.05, GraphPad Software Inc, San Diego, Calif.

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