

# Applicability of thyroxine measurements and ultrasound imaging in evaluations of thyroid function in turtles

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## Abstract

**Introduction:** The thyroid and parathyroid glands play a major role in maintaining physiological homeostasis in all vertebrates. Reptiles have plasma concentrations of thyroid hormones far lower than mammals. Low levels of these hormones in reptiles impede thyroid hormone detection with assays designed for the higher levels of mammals. The aim of this study was to explore teaming this with ultrasound imaging of the thyroid to appraise glandular function. **Material and Methods:** Thyroid function of four pond sliders was evaluated based on the results of T4 analyses and ultrasound. **Results:** The concentrations of T4 varied considerably between the examined animals from <9 nmol/L to >167.3 nmol/L. Ultrasound examination revealed uniform echogenicity and a smooth outline of the thyroid gland in all animals. **Conclusion:** Monitoring of thyroid function based on T4 and electrolyte concentrations is helpful in assessing the health and living conditions of reptiles, which is important in veterinary practice but problematic. Ultrasound examinations are useful in diagnosing changes in gland structure, such as tumours and goitres, and a combination of both methods supports comprehensive assessments of the anatomy and function of the thyroid gland.

**Keywords:** reptiles, thyroid gland, ultrasound, thyroxine.

## Introduction

The thyroid is one of the major glands of internal secretion in both endothermic and ectothermic animals. Mammals have a double-lobed thyroid gland, whereas in turtles the thyroid gland is a single-lobed structure (16) which is located on the ventral side of the trachea and near the base of the heart. It consists of follicular cells that produce and release thyroid hormones which are identical in turtles and other vertebrates (12). Thyroid hormones play a key role in carbohydrate, fat and protein metabolism, and they participate in the regulation of other hormones. The thyroid gland is a major factor in maintaining physiological homeostasis in all vertebrates. In reptiles, the thyroid gland performs a function in ecdysis, reproduction, tail regeneration, growth, endocrine function, and metabolic rate (18). Thyroid hormone deficiency (hypothyroidism) and excessive production of the

thyroid hormone (hyperthyroidism) exert harmful effects.

The main thyroid hormones are triiodothyronine (T3) and thyroxine (T4), which in reptiles, participate in growth, development, reproduction, and skin shedding. They also play an important role in reptilian metabolism and behaviour (19). In most reptile species T4 levels are higher than T3. Several turtle genera (*Chrysemys*, *Pseudemys*, and *Trachemys*) have much higher thyroid values than other reptiles, which may be due to the presence of a thyroxine binding protein (TBP) (3). Thyroid hormones bind with receptors in cell nuclei and mitochondria to activate enzymes responsible for glycolysis and ATP production, which boosts metabolism in target organs (2, 19). Those hormones also influence peripheral tissues by increasing energy and oxygen consumption, raising the heart rate, increasing blood pressure, and stimulating the sympathetic nervous system (19). Thyroxine

increases the affinity and efficacy of proteins that bind and transport vitamin D<sub>3</sub> (14). It invigorates the transport of vitamin D<sub>3</sub> and its metabolites from the skin and intestines to the liver where these compounds undergo hydroxylation at position C-25, the first reaction in calcitriol synthesis. These processes are essential for mineral metabolism in turtles. Vitamin D<sub>3</sub> regulates calcium homeostasis and is necessary for calcium absorption in the intestines and for inducing the synthesis of calbindin, a calcium-binding protein. It also makes intestinal mucosal cells more permeable to calcium (22). Thyroid hormones serve an important function in the early stages of egg incubation. In common snapping turtle (*Chelydra serpentina*) hatchlings a significant negative correlation was reported between incubation temperature and plasma levels of thyroxine which induces parallel changes in the resting heart rate of hatched animals. This finding could be important in the evolution of temperature-dependent sex determination (17).

The thyroid gland is rarely examined during routine medical examinations in turtles. Unlike in reptiles, various methods for diagnosing thyroid disorders have been developed in mammals. The concentrations of TSH, free T4 (fT4), free T3 (fT3), and antithyroid peroxidase autoantibodies (anti-TPO) can be determined during analyses of human blood (5). The range of laboratory tests available for companion animals such as dogs, includes analyses of the concentrations of T4, fT4, T3, TSH, parameter K, thyroglobulin antibodies, and T4 antibodies, and the TRH stimulation test (9). In reptiles, the available options for analysing thyroid parameters are very limited. Reptiles have plasma concentrations of thyroid hormones far lower than mammals. Low levels of these hormones in reptiles impede thyroid hormone detection with assays designed for the higher levels of mammals. T4 and T3 values in reptiles are roughly 20% and 25%, respectively, of the average values in mammals. T4 has the highest concentration (except in the Indian cobra *Naja naja* and Indonesian beauty snake *Elaphe taeniuria*), followed by T3, fT3, and fT4. In *Testudo* spp. fT4 is often below the limits of detection (<0.45 pmol/L) of mammalian thyroid hormone assays (3).

The thyroid gland can be effectively evaluated in an ultrasound examination. As the structure of the thyroid gland is similar in all vertebrates, the difficulty with which an ultrasound test is performed in turtles is determined by anatomical features which differ between species. For example, tortoises and turtles of the genera *Testudo* and *Trionyx* (softshells) are characterised by relatively wide acoustic windows (the carotid and femoral triangles), whereas the Horsfield's tortoise (*Testudo horsfieldii*) and the red-footed tortoise (*Chelonoidis carbonarius*) have narrow acoustic windows which pose diagnostic problems during ultrasound examinations. Turtles without dermal bones and species with incompletely ossified shells, such as

softshell turtles, are scanned through the plastron. Most turtles can undergo an ultrasound examination without sedation and patients can be effectively immobilised with the help of an assistant. The turtle is placed on a supporting structure to prevent the limbs from touching the ground, and to prevent the animal from moving. If the patient is less willing to cooperate, the head or front limb may have to be immobilised for better visibility (23).

The transducer is placed directly on the skin and coupling gel is used. Turtles can also be scanned in water, and this option is used to examine very small animals and weak sea turtles. Probes not designed for underwater use can be placed inside an examination sleeve. The appropriate area can also be scanned through a glove filled with water. Species with deep and narrow acoustic windows (such as *Terrapene* spp.) can be positioned with the acoustic window facing upwards during the examination. As previously noted as the general technique, the region is covered with coupling gel and scanned with a transducer. Turtles such as *Pseudemys* spp., *Trachemys* spp., and soft-shelled turtles (*Trionyx* spp.) can be partially submerged in water during an ultrasound examination. The thyroid gland of these species is examined through the cervicobrachial acoustic window (23).

The aim of this study was to assess thyroid function of captive turtles based on the results of T4 analyses and ultrasound scans. The applicability of these analytical methods for monitoring the condition of turtles was evaluated.

## Material and Methods

The thyroid function of four pond sliders (*T. scripta*) comprising two red-eared slider (*Trachemys scripta elegans*) females, one yellow-bellied slider (*T. scripta scripta*) female, and one Cumberland slider (*T. scripta troostii*) male was evaluated. The animals were characterised by similar body weights ranging from 0.922 to 1.546 kg. They were kept in aquaterraria equipped with basking platforms with heating and UVB lamps (25 W, 10%), and provided with a light/dark cycle of 13/11 and varied temperatures (Table 1). Due to a burned-out light bulb one of the red-eared sliders was kept in a terrarium without a basking spot for a short period. Turtles were fed every other day with commercially formulated diet. Blood samples for T4 analysis were collected in a minimum amount of around 1 mL from the *vena jugularis dorsalis* (Fig. 1) into 4.5 mL VACUETTE tubes containing up to 0.4 mL/100 g of serum clot activator. EIA analyses were performed by IDEXX Laboratories (UK). Blood was sampled twice at an interval of 19 days. To evaluate thyroid gland activity T4 level was measured and biochemical parameters such as aspartate transaminase (AspAT), alanine transaminase (ALAT), cholesterol, calcium,

phosphorus, sodium, potassium, and chloride levels were measured to assess overall condition and health.



Fig. 1. Puncture site

Turtles were subjected to an ultrasound examination with the Dramiński 4Vet ultrasound scanner (Poland) with microconvex probes of diameter 2.2 cm and 3 cm. The ultrasound scans were carried out using 8MHz and a depth setting of 70–80 mm. The turtles were not sedated but restrained manually with the help of an assistant. The thyroid gland was scanned through the right and left cervicobrachial acoustic window (Fig. 2).



Fig. 2. Left cervical-brachial window

## Results

The concentrations of T4 varied considerably between the examined animals from <9 nmol/L to >167.3 nmol/L, as presented in Table 1 along with

other investigated parameters. The EIA method conducted by IDEXX Laboratories is not accredited for this species and the results should be interpreted with caution. There are no commercially available tests designed for turtles.

The results of thyroid gland measurements taken during ultrasound examination are presented in Table 2. During the ultrasound examination, visible changes were not observed in the structure of the thyroid glands which were characterised by uniform echogenicity and a smooth outline (Figs 3 and 4).



Fig. 3. Ultrasound image of *T. scripta elegans*



Fig. 4. Ultrasound image of *T. scripta elegans*

Biochemical blood parameters were within the norm in all animals, excluding cholesterol levels in one *T. scripta elegans* female. The results were interpreted on the basis of standards given by Carpenter et al. (4). Turtles were in good clinical condition, with no changes in behaviour or appetite observed.

**Table 1.** T4 concentrations in the examined turtles in view of ambient conditions, patient weight and size

Species		<i>T. scripta elegans</i>	<i>T. scripta elegans</i>	<i>T. scripta scripta</i>	<i>T. scripta troostii</i>
Gender		Female	Female	Female	Male
Body weight (kg)		1.484	1.546	1.482	0.922
Length of carapace/plastron (cm)		18.5/17.5	21/19.9	19.5/19.3	18/16.2
Presence of a member of the opposite sex		-	-	+	+
03.02	Air temperature	21.6	26.3	26.1	26.1
	Water temperature	18	21.2	20.7	20.7
	T4 (nmol/L)	< 9.0	> 167.3	112.0	150.6
22.02	Air temperature	26.6	26.3	26.1	26.1
	Water temperature	20	21.2	20.7	20.7
	T4 (nmol/L)	38.6	54.1	139.0	97.8
	Cholesterol (mmol/L)	9.351	4	5.498	4.125
	AspAT (u/L)	171	97	91	154
	ALAT (u/L)	4	2	11	13
	Calcium (mmol/L)	4.47	2.78	2.81	2.4
	Phosphorus (mmol/L)	1.38	0.74	1.21	0.94
	Blood gasometry at 37°C				
	Sodium (mmol/L)	134	127	127	131
	Potassium (mmol/L)	3.06	3.84	4.26	4.23
	Chloride (mmol/L)	98	99	100	99

**Table 2.** Length and width of the thyroid gland in ultrasound examinations (mm)

	Mean	<i>T. scripta elegans</i>	<i>T. scripta elegans</i>	<i>T. scripta scripta</i>	<i>T. scripta troostii</i>
Length (mm)	11.23	10.4	11.2	12.2	11.1
Width (mm)	8.95	9.0	10.0	9.2	7.6

## Discussion

The thyroid gland has numerous functions, but its main role is to control energy consumption under supportive environmental conditions. This gland, the pituitary, and the hypothalamus as an axis are stimulated by nutrient absorption (16, 19). The thyroid gland is more active in periods of heightened metabolic activity, such as growth and reproduction, and it is less active in periods of low metabolic activity, including hibernation. In reptiles, thyroid activity is temperature-dependent (11). The lowest T4 concentration of <9 nmol/L was observed in the animal kept in a terrarium without a heating lamp, due to a burned-out light bulb. When a heating lamp was placed inside the terrarium, T4 levels increased to 38.6 nmol/L, which could confirm the hypothesis that the secretion of the hormone is temperature-dependent. A comparison of thyroid parameters in reptiles and mammals revealed that the reptilian thyroid gland is more active at higher temperatures, but it is always less active than the mammalian counterpart. A thyroidectomy led to a considerable drop in the metabolic rate of animals kept at 30–32°C, whereas thyroxine injections significantly intensified metabolic processes at the same temperature (11). Seasonally influenced plasma thyroxine levels in *Gopherus agassizii* were observed (13). Thyroxine concentration was lowest during

hibernation and began to increase upon waking. In females, thyroxine production peaked in early spring, whereas males showed two T4 peaks in early spring and late summer. The second peak is associated with the mating season, during which males compete for territory and access to females (13).

Thyroid hormone levels are also sex-dependent. In *T. scripta*, thyroxine levels are usually higher in females (176.35 ± 22.4 nmol/L) than in males (108 ± 17.76 nmol/L) (15). Our results do not confirm these findings as T4 levels in members of both sexes were similar. In the examined male, the drop in T4 levels on the second sampling date could be indicative of the end of the mating season. In the *T. scripta elegans* female, the high concentration of T4 (> 167.3 nmol/L) on the first sampling date and the significant drop to 54.1 nmol/L on the second sampling date are difficult to explain. The observed change could be attributed to a T4 peak linked with the mating season (13). The absence of male turtles and copulation could have induced the end of mating and, consequently, a decrease in the amount of secreted T4. This observation could indicate that season affects T4 secretion. The concentrations of thyroid hormones also vary between species. The reference values for thyroid hormones in different species of turtles and tortoises, and one lizard and snake species are given in Table 3.

**Table 3.** Reference values for thyroid hormones

	Species	T <sub>3</sub> (nmol/L)	T <sub>4</sub> (nmol/L)	Reference	
Turtles	Painted turtle ( <i>Chrysemys picta</i> )		102.96	13	
	Red-eared slider ( <i>Trachemys scripta elegans</i> ) female		146.33–191.11	15	
	Red-eared slider ( <i>Trachemys scripta elegans</i> ) male		90.22–125.74	15	
	Red-eared slider ( <i>Trachemys scripta elegans</i> )	0.15	12.87	10	
	Red-eared slider ( <i>Trachemys scripta elegans</i> ) young turtles		123.04–167.04	10	
	Red-eared slider ( <i>Trachemys scripta elegans</i> ) young turtles		10.68–69.88	21	
	Indian flapshell turtle ( <i>Lissemys punctata punctata</i> )		102.76–108.23	20	
	Eastern long-necked turtle ( <i>Chelodina longicollis</i> )	0.28–0.31	0.55–0.69	11	
	Tortoises	Horsfield's turtle ( <i>Testudo horsfieldii</i> )	0.93–1.38	6.3–11.46	1
		Desert tortoise ( <i>Gopherus agassizii</i> ) female		0.51–3.6	13
		Desert tortoise ( <i>Gopherus agassizii</i> ) female		0.46–3.22	13
	Lizards	Green iguana ( <i>Iguana iguana</i> )		2.97–4.65	8
Snakes	Ball python ( <i>Python regius</i> )		0.93–4.79	7	

Apart from temperature and season, T<sub>4</sub> levels are also influenced by feed intake. A considerable drop in T<sub>4</sub> concentration was reported in *G. agassizii* turtles deprived of feed for several weeks. In these animals, T<sub>4</sub> levels increased within 36 h after feeding (13). Hormones secreted by other endocrine glands also influence thyroid activity. It was noted that in the Indian flapshell turtle (*Lissemys punctata*), melatonin inhibited thyroid activity. When administered to turtles, melatonin decreased body weight, size of follicular endothelial cells, and plasma concentrations of thyroid peroxidase and thyroxine. Melatonin probably exerts inhibitory effects by influencing the release of thyrotropin from the pituitary and activity of the thyroid gland in turtles (20). Hypothyroidism/hypoidinism is the most commonly diagnosed disease of the thyroid gland in goitrogen-fed captive turtles (16). In the present study's results the same light/night cycle and diet were experienced by all the subjects, and so these parameters probably did not have any significant influence. Other thyroid diseases affecting turtles include adenoma and adenocarcinoma (16).

During ultrasound examinations, the visibility of the thyroid gland is determined by the degree of neck extension. The thyroid gland is localised medially to the ventral side of the trachea and adjacent to the heart base. The region had the highest visibility when scanned through the cervical-brachial acoustic window. The thyroid gland is an oval, teardrop-shaped, hyperechogenic organ. It is caudally limited by hypoechogenic jugular arteries on both sides. During the ultrasound examination, visible changes were not observed in the structure of the thyroid gland which was characterised by uniform echogenicity and a smooth outline.

The examined thyroid glands had a length of 10.4–12.2 mm and a width of 7.6–10.5 mm. Our results are similar to those noted in turtle species inhabiting the Mediterranean region (length 8–14 mm, width 6–13 mm) (23). This is an interesting observation because the T<sub>4</sub> levels of those Mediterranean species, evaluated as adults as were the turtles in the present study, were completely different to the levels obtained in our research although all turtles were of similar size living under optimal conditions. This suggests lack of correlation between the size of the thyroid gland and hormone levels.

The most common thyroid gland diseases include goitre, hypothyroidism, and hyperthyroidism. Goitre is the enlargement of the thyroid gland in an attempt to compensate for a deficiency in iodine, and its main clinical symptom is swelling cranial to the heart. Evaluation of thyroid gland measurements with the use of ultrasound together with the T<sub>4</sub> test can be used to diagnose goitre. Hypothyroidism is rather uncommon in reptiles. It is characterised by decreased production and release of thyroid hormones resulting in decreased metabolic activity. The most common presenting signs are anorexia, lethargy, goitre, and myxoedema of subcutaneous tissues generally involving the head, neck, and proximal forelimbs. Clinical signs may be suggestive of hypothyroidism, but proper diagnosis should be based on measurements of total T<sub>4</sub>. Hyperthyroidism is a multisystemic disorder resulting from elevated levels of T<sub>3</sub> and T<sub>4</sub>, which leads to a state of abnormally high metabolism. It is assumed that this condition is under-diagnosed in reptiles. Literature is very scarce but there are two reports describing this disease in reptiles. The first one concerns a green iguana (*I. iguana*) with signs of

polyphagia, weight loss, loss of dorsal spines, hyperactivity, increased aggression, and a palpable mass in the ventral cervical region. Diagnosis of hyperthyroidism was based on an elevated T4 level. Surgical thyroidectomy resulted in return to normal appetite, activity levels, weight, and heart rate, and in regrowth of dorsal spines (8). The second case describes a leopard gecko (*Eublepharis macularius*) presenting with signs of anorexia, diarrhoea, and more frequent skin shedding (from monthly to every two weeks). Hyperthyroidism was determined by elevated total T4 levels (3).

Neoplasia of the endocrine organs, especially of the thyroid gland, is rare in reptiles, although thyroid carcinomas and adenomas have been reported in turtles and lizards (6). There is also one report describing papillary carcinoma in the thyroid gland of a red-eared slider (6). Using ultrasound examination can be helpful in diagnosing such disease conditions.

These examples show instances for potential implementation of the described evaluation methods and should encourage veterinarians to pursue diagnoses of thyroid diseases.

The noted variations in T4 levels can probably be attributed to species/subspecies-specific differences as well as variations in habitat conditions (no heating lamp or presence of a member of opposite sex). The results of this study suggest that the health of the thyroid gland is difficult to evaluate based on analyses of T4 levels alone. The determination of reference values for T4 concentrations in turtles poses a challenge because those values are influenced by numerous factors, including temperature, sex, and diet. For this reason, it is difficult to compare the results of laboratory analyses in reptiles with those in dogs and cats. Our results indicate that monitoring of thyroid function based on T4 and electrolyte concentrations is helpful in assessing the health and living conditions of reptiles, to do which is an important need in veterinary practice but a problematic undertaking. Ultrasound examinations are useful in diagnosing changes in the gland structure, such as tumours and goitres, and a combination of both methods supports comprehensive assessments of the anatomy and function of the thyroid gland. In view of the current state of knowledge, evaluations of the reptilian thyroid gland can produce reliable results when analyses of T4 levels are combined with ultrasound imaging.

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## References

- Balletto E., Cherchi M.A., Melodia F., Mensi P.: Temperature-dependent thyroid hormone biosynthesis in *Testudo horsfieldi* Gray. *Gen Comp Endocrinol* 1979, 39, 548–561.
- Bassett J.H.D., Harvey C.B., Williams G.R.: Mechanisms of thyroid hormone receptor-specific nuclear and extra nuclear actions. *Mol Cell Endocrinol* 2003, 213, 1–11.
- Boyer T.H., Steffers Z.J.: Reptilian thyroid anatomy, physiology, and disease. *Proc Assoc Reptilian Amphibian Veterinarians* 2011, pp. 18–35.
- Carpenter J.W., Mashima T.Y., Rupiper D.J.: *Exotic Animal Formulary*, Saunders, Saint Louis, USA, 2005, p. 108.
- Dayan C.M.: Interpretation of thyroid function tests. *Lancet* 2001, 357, 619–624.
- Gál J., Csikó G., Pásztor I., Böleskey-Molnár A., Albert M.: First description of papillary carcinoma in the thyroid gland of a red-eared slider (*Trachemys scripta elegans*). *Acta Vet Hung* 2010, 58, 69–73.
- Greenacre C.B., Young D.W., Behrend E.N., Wilson G.H.: Validation of a novel high-sensitivity radioimmunoassay procedure for measurement of total thyroxine concentration in psittacine birds and snakes. *Am J Vet Res* 2001, 62, 1750–1754.
- Hernandez-Divers S.J., Knott C.D., MacDonald J.: Diagnosis and surgical treatment of thyroid adenoma induced hyperthyroidism in a green iguana (*Iguana iguana*). *J Zoo Wildl Med* 2001, 32, 465–475.
- <https://www.idexx.com/small-animal-health/directory-tests-services.html> (accessed 10.01.2018)
- Hugenberger J.L., Licht P.: Characterization of thyroid hormone 5'-monodeiodinase activity in the turtle (*Trachemys scripta*). *Gen Comp Endocrinol* 1999, 113, 343–359.
- Hulbert A.J., Williams C.A.: Thyroid function in a lizard, a tortoise and a crocodile, compared with mammals. *Comp Biochem Physiol A Comp Physiol* 1988, 90, 41–48.
- Jacobson E.R.: Overview of reptile biology, anatomy, and histology. In: *Infectious Diseases and Pathology of Reptiles: Color Atlas and Text*, edited by Jacobson E.R., CRC Press, Taylor & Francis Group, Boca Raton, USA, 2007, pp. 19–20.
- Kohel K.A., MacKenzie D.S., Rostal D.C., Grumbles J.S., Lance V.A.: Seasonality in plasma thyroxine in the desert tortoise, *Gopherus agassizii*. *Gen Comp Endocrinol* 2001, 121, 214–222.
- Licht P.: Thyroxine-binding protein represents the major vitamin D binding protein in the plasma of the turtle, *Trachemys scripta*. *Gen Comp Endocrinol* 1994, 93, 82–92.
- Licht P., Denver R.J., Stamper D.L.: Relation of plasma thyroxine binding to thyroidal activity and determination of thyroxine binding proteins in a turtle, *Pseudemys scripta*. *Gen Comp Endocrinol* 1990, 80, 238–256.
- McArthur S., Meyer J.: Endocrine system. In: S. McArthur, R. Wilkinson, J. Meyer (eds): *Medicine and Surgery of Tortoises and Turtles*. Wiley-Blackwell, 2004, 71–72.
- O'Steen S., Janzen F.J.: Embryonic temperature affects metabolic compensation and thyroid hormones in hatchling snapping turtles. *Physiol Biochem Zool* 1999, 72, 520–533.
- Rivera S., Lock B.: The reptilian thyroid and parathyroid glands. *Vet Clin North Am Exot Anim Pract* 2008, 11, 163–175.
- Rostal D.C., McCoy E.D., Mushinsky H.R.: *Biology and Conservation of North American Tortoises*. Johns Hopkins University Press, Baltimore 2014, pp. 93–94.

20. Sarkar S., Sarkar N.K., Bhattacharyya S., Das P.: Melatonin action on thyroid activity in the soft-shelled turtle, *Lissemys punctata punctata*. *Folia Biol (Krakow)* 1997, 45, 109–112.
21. Stamper D.L., Denver R.J., Licht P.: Effects of thyroidal status on metabolism and growth of juvenile turtles, *Pseudemys scripta elegans*. *Comp Physiol* 1990, 96A, 67–73.
22. Wail P.A.: The diversity of the endocrine system. In: R.K. Murray, D.K. Granner, V.W. Rodwell (eds): *Harper's Illustrated biochemistry*, McGraw-Hill Education, LANGE, New York City, USA, 2015, pp. 507–511.
23. Wilkinson R., Hernandez-Divers S., Lafortune M., Calvert I., Gumperberger M., McArthur S.: Diagnostic imaging techniques. In: S. McArthur, R. Wilkinson, J. Meyer (eds): *Medicine and Surgery of Tortoises and Turtles*, Wiley-Blackwell, Oxford, U.K., 2004, pp. 187–190.