A comparison of thyroid hormone levels and plasma capillary zone electrophoresis in red-eared sliders (Trachemys scripta elegans) and map turtles (Graptemys spp.) depending on season and sex

Christoph Leineweber1,2 | Sabine Öfner3 | Anke C. Stöhr4 | Rachel E. Marschang1 | Karina Mathes2

1Laboklin GmbH & Co. KG, Bad Kissingen, Germany
2Clinic for Small Mammals, Reptiles and Birds, University of Veterinary Medicine Hannover, Hannover, Germany
3Reptile Rescue Center Munich e.V., Munich, Germany
4Department of Veterinary Clinical Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA, USA

Correspondence
Christoph Leineweber, Laboklin GmbH & Co. KG, Steubenstrasse 4, 97688 Bad Kissingen, Germany.
Email: christoph.leineweber@tiho-hannover.de

Funding information
Wild Animal Health Fund, American Association of Zoo Veterinarians (AAZV); Association of Reptilian and Amphibian Veterinarians (ARAV); Deutsche Gesellschaft für Herpetologie und Terrarienkunde (DGHT)

Abstract

Background: Thyroid hormones and capillary zone electrophoresis are rarely studied in reptile medicine.

Objectives: The goal of this prospective study was to establish RIs for plasma proteins and thyroid hormones, total tetraiodothyronine (tT4), free T4 (fT4), total triiodothyronine (tT3), and free T3 (fT3), as well as iodine blood levels in red-eared sliders (Trachemys scripta elegans) and map turtles (Graptemys spp.).

Methods: Variables were measured over 1 ½ years to determine variations depending on sex and season, as well as any correlations between the parameters. A total of 131 blood samples from red-eared sliders and 51 blood samples from map turtles were collected from May 2016 to November 2017. The levels of tT4, fT4, and fT3 were measured using standardized autoanalyzer test systems; tT3 was measured by ELISA and iodine inductively coupled plasma mass spectrometry. Total protein was quantitated using the biuret method, and plasma protein fractions were measured using capillary zone electrophoresis.

Results: The results showed significant variations in thyroid hormone levels and plasma protein fractions depending on sex and season. Total T4 and fT4 concentrations were significantly (P < .05) correlated in both turtle species and sexes in all seasons. Thyroid hormone levels correlated with blood proteins in a few seasons, including a positive correlation between tT4 and fT4 in female red-eared sliders in fall and female map turtles in summer and fall.

Conclusions: This study demonstrates the importance of considering species, season, and sex variations when interpreting thyroid hormone and plasma protein levels. It also shows that thyroid hormone levels are not always influenced by total protein and albumin.

KEYWORDS
albumin, iodine, tetraiodothyronine, total protein, tT3, tT4
1 | INTRODUCTION

The thyroid gland is present in all vertebrate species, and the structure and types of hormones produced show minimal variation between the different reptile species. However, the function and physiologic levels of the thyroid hormones have been studied in less detail in reptiles compared with some mammal species. Thyroid hormones play an important role in metabolism, and influence a wide range of physiologic functions, including metabolic rates, growth, regeneration, ecdysis, the production of other endocrine hormones, and reproduction. The thyroid gland is influenced by multiple external factors including, diet, season, temperature, light cycle, and iodine supply. At this time, RIs for thyroid hormone levels in chelonians have been calculated for several species of terrestrial tortoises, sea turtles, and freshwater turtles. A majority of these studies are limited to tT4 levels and have not been differentiated between the sexes and seasons from which the sample was collected. Licht et al showed seasonal variations in the tT4 levels of free-living painted turtles in Michigan but did not differentiate between the sexes of the turtles.

Another important facet influencing thyroid hormone levels in the blood is that these hormones bind to several different transport proteins in the blood system. The binding activity and binding capacity of albumin, and the thyroxin-binding protein (TBP) are, however, very different between the various chelonian species. Pavgi et al showed that TBP concentrations were very different between juvenile and adult red-eared sliders (Trachemys scripta elegans), and TBP levels in adult turtles were higher than in juvenile females. In addition, adult females were shown to have significantly higher TBP levels compared with those of adult males. A correlation between TBP concentrations and tT4 binding has been demonstrated. Licht et al showed that distinct changes in tT4 binding lead to changes in plasma T4 concentrations. Similarly, TBP concentrations were shown to be regulated by the concentration of circulating tT4; for instance, lower tT4 levels lead to higher TBP levels. The levels of total protein (TP) and, therefore, the levels of albumin and TBP also varied between individual turtles. Glennemeier et al reported that the percentages of albumin and TBP in the blood of sliders (T scripta) and snapper turtles (Chelydra serpentine) are related to the percentage of bound tT4 and unbound free T4 (fT4), but only the free forms are bioactive. This indicates that the total blood protein and composition of the blood protein fractions, play an important role in thyroid hormone levels.

Thyroid disease has been described in individual case reports. However, the lack of RIs and standardized testing have complicated diagnoses in such cases, highlighting the need for further studies.

Both red-eared sliders and map turtles are native to North America, where they are abundant. These turtles are also commonly kept as pets in North America and other parts of the world. Due to illegal introduction into the wild, these species are also commonly found in freshwater habitats of Europe. To lower the impact on native herpetofauna, trading red-eared sliders has been made illegal in Europe, but many are still kept as pets, which remains legal. These species, therefore, play an important role in conservation and husbandry.

The goal of this study was to measure plasma protein fractions and thyroid hormone levels for tT4, fT4, tT3, free T3 (fT3), and iodine in captive red-eared sliders (T s elegans) and map turtles (Graptemys spp.) to obtain initial data on the levels and, where possible, to establish RIs, including the evaluation of changes in different seasons. We also aimed to compare the values measured in two different genera of emydid turtles. We hypothesized that the plasma protein fractions and thyroid hormone levels varied between the seasons, sex, and turtle species and that variations in plasma protein and iodine levels influence thyroid hormone levels. It was also hypothesized that there would be a correlation between the levels of the different thyroid hormones.

2 | MATERIALS AND METHODS

2.1 | Blood collection and sample preparation

Blood samples from red-eared sliders and map turtles were collected from May 2016 to November 2017. The red-eared sliders and map turtles had body weights between 384 and 2867 g and between 170 and 958 g, respectively. Some of the turtles were sampled repeatedly for the study at different times of the year; others were opportunistically included when blood was collected for routine health screening. Repeat blood collection for this study was carried out under animal welfare permit number TVA-2017-V-18 from the animal welfare committee of the University of Veterinary Medicine, Hannover, accepted on the March 16, 2017. The turtles were kept in two reptile rescue centers in Germany. They were housed in greenhouses with naturalistic indoor ponds, various aquatic plants, and littoral zones with sand for oviposition. The light and temperature conditions were dependent on the natural conditions, but extremes in temperature were mitigated by the greenhouses. The turtles were all fed a varied diet of pellets, small fish, crabs, cuttlebone, various aquatic insects, lettuce, water plants, and wild herbs such as dandelion, field bindweed, and plantains. At the time of blood collection, all turtles were given a general health check (which included an examination of general health, nutritional and care status, the nares, eyes, oral cavity including the mucous membranes, skin, extremities, tail, cloaca, and the form and strength of the shell, as well as a fecal exam) and were all considered clinically healthy. Blood samples were collected from the dorsal coccygeal vein and, in some cases, from the subcarapacial sinus. Lithium heparinized blood and serum samples were collected from red-eared sliders (135 plasma and 127 serum samples) and map turtles (52 plasma and 45 serum samples). Samples with visible lymph dilutions were not included in the study. Blood was transferred into lithium heparinized and serum tubes and stored at 8°C. For thyroid hormone levels and other plasma variables, the samples were centrifuged no later than 4 hours after collection. The plasma was sent over-night to the laboratory and analyzed 1 day after collection. To eliminate samples with lymph contamination and exclude turtles...
with inflammatory processes from the study, PCVs were measured using microhematocrit capillary tubes that were centrifuged for 5 minutes at 12 000g (Haemofuge, Heraeus Sepatech) and blood smears were microscopically evaluated. The smears were prepared immediately after blood collection, air-dried, and stained using Diff-Quick (Labor + Technik Eberhard Lehmann GmbH). Leukocytes were immediately after blood collection, air-dried, and stained using Diff-Quick (Labor + Technik Eberhard Lehmann GmbH). Leukocytes were calculated at 400× magnification in 10 fields. For the differential blood count, 100 cells were evaluated at 1000× magnification. Samples with leukocyte counts over 19.4 × 10⁹/µL or with PCVs under 0.10 L/L were excluded from the statistical analyses.

### 2.2 Measurement of thyroid hormone levels, iodine, and plasma protein fractions

The tT4 was measured using an IMMULITE 2000 Xpi Immunoassay-system canine T4 (KT4) (Siemens Healthcare GmbH) and a cobas 8000 analyzer series module e602 T4 (Roche Diagnostics), the fT4 was measured using a cobas 8000 analyzer series module e602 FT4 II (Roche Diagnostics), and the tT3 was measured using an ADVIA Centaur Immunoassay-system ADVIA Centaur FT3 Assay (Siemens Healthcare GmbH). The samples for tT3 and iodine analyses were stored at −20°C and analyzed 1 month to 1 year after collection. Total T3 was measured using the Triiodothyronine (T3) ELISA Kit (Biomatik Life Science Products and Services). Some of the tT4 and FT4 levels measured in the turtles, especially in samples collected during the summer, were over the detection limit of the tests. The tT4 detection limit of the canine T4 from IMMULITE 2000 Xpi is 15 µg/dL. The samples in which higher concentrations were measured were retested using the tT4 test of the cobas 8000 with a detection limit of 24 µg/dL. The detection limit of the tT4 test was 100 pmol/L. Samples with higher levels were diluted 1:10 with a physiologic saline solution (0.9% NaCl) and measured again. The test systems used for the measurement of thyroid hormones were validated for the red-eared slider turtle, no samples were collected in winter. Some of the samples collected in the fall, and some of the samples collected in the summer were under the detection limit of the tT4 test of <0.12 µg/dL. For statistical analyses, these results (one red-eared slider in the summer and three in the fall, one map turtle in the summer and 10 in the fall) were set at half of the cut-off (0.06 µg/dL) for the calculation of the RIs. Statistical analyses were carried out using the statistical analysis software (SAS) (SAS Institute Inc) according to the recommendations of the ASVCP guidelines for RIs. The histogram, Q-Q plots, and Shapiro-Wilk test, as well as the evaluation of whether all values were within two SDs of the mean, were used as criteria to determine normal distributions. The values that were not normally distributed (P < .05) are marked in Tables 1-4, and the RIs for these parameters were calculated using the nonparametric method (10th-90th percentiles). The number of samples was not sufficient for the calculation of the 10th and 90th percentile according to these guidelines at all sample times and, in these cases (<20 samples), only the mean, SD, median, and minimum and maximum values were calculated. For the use of the ANOVA mixed models and t-test, data were normalized by logarithmic transformation. For the calculation of statistical effects, an ANOVA mixed model (SAS) was used, a value of P < .05 was considered the cut-off for significance. The differences between the two detection methods for albumin were calculated using a t-test. A Spearman test (SAS) was used for the calculation of the correlation effects between thyroid hormone levels, iodine, and TP concentrations.

### 3 RESULTS

#### 3.1 Validation of test systems

All thyroid hormones (tT4, fT4, tT3, and fT3) showed linear regression within the limits of detection provided by the manufacturer. The short-term replication showed an SD of 0.25 and a CV of 0.08 for the tT4 test on the cobas and an SD of 0.08 and a CV of 0.03 for the IMMULITE tT4 test. The FT4 test showed an SD of 2.49 and a CV of 0.04, and the fT3 test showed an SD of 0.11 and a CV of 0.20. The long-term replication showed an SD of 0.38 and...
| Parameter       | Unit     | Spring | Summer | Fall                      |
|-----------------|----------|--------|--------|---------------------------|
|                 |          | Mean   | SD     | Median | Min | Max | Mean | SD | Median | Min | Max | 10% Percentile | 90% Percentile |
| tT4 µg/dL       |          | 8.5±  | 6.50   | 5.10  | 23.30 | 6.27± | 6.54 | 3.20 | 0.10   | 24.32 | 0.40 | 0.40 | 14.60          |
| fT4 pmol/L      |          | 113.6± | 144.72 | 58.80 | 443.40 | 93.11± | 108.08 | 38.00 | 4.00   | 434.60 | 6.90 | 6.90 | 200.80         |
| tT3 ng/mL       |          | 0.46±  | 1.88b  | 0.43  | 4.32  | 3.91± | 4.35 | 1.46 | 0.28   | 13.14 | 0.41 | 0.41 | 10.36          |
| fT3 pmol/L      |          | 0.4±   | 1.94b  | 1.09  | 3.80  | 0.73± | 0.58 | 0.70 | 0.00   | 2.10  | 0.00 | 0.00 | 1.50           |
| n               | 1        | 10     | 23     |        |       |      |      |      |        |       |      |      |                |
| Iodine µg/L     |          | 67.8±  | 100.59b | 59.16 | 98.50 | 28.10 | 185.20 | 49.75± | 31.38 | 41.80 | 16.90 | 131.0 | 18.70 | 84.30          |
| n               | 1        | 7      | 23     |        |       |      |      |      |        |       |      |      |                |
| Total protein g/L |         | 33.5±  | 31.0b  | 13.1  | 28.4  | 13.5  | 51.6  | 45.3± | 11.4  | 44.7  | 24.0  | 64.9  | 32.0  | 60.6           |
| Albumin (BCG) g/L |         | 12.4±  | 9.5b   | 8.4   | 7.8   | 0.4   | 21.7  | 17.7± | 4.8   | 17.5  | 8.1   | 25.6  | 11.2  | 24.4           |
| Albumin total (CZE) g/L | | 8.7±  | 9.6a   | 6.4   | 6.8   | 3.6   | 21.6  | 12.3± | 3.1   | 12.1  | 5.8   | 19.9  | 9.0   | 15.4           |
| Albumin total (CZE) % |   | 26.0±  | 29.0b  | 7.8   | 27.7  | 16.4  | 44.7  | 27.2± | 4.7   | 26.2  | 15.5  | 37.9  | 22.0  | 32.5           |
| Prealbumin (CZE) % |          | 6.5±  | 7.1b   | 4.5   | 5.7   | 2.3   | 15.7  | 4.5±  | 1.9   | 4.0   | 2.3   | 9.7   | 2.6   | 7.3            |
| Albumin (CZE) % |          | 19.5±  | 21.9b  | 6.2   | 23.1  | 11.3  | 30.4  | 22.7± | 4.3   | 23.1  | 12.2  | 30.2  | 18.0  | 28.2           |
| A:G ratio %     |          | 0.35±  | 0.43b  | 0.17  | 0.39  | 0.20  | 0.81  | 0.38± | 0.09  | 0.36  | 0.18  | 0.61  | 0.28  | 0.48          |
| α-globulins %   |          | 31.1±  | 33.6b  | 6.2   | 34.5  | 23.1  | 41.9  | 33.0± | 4.2   | 32.3  | 25.1  | 39.8  | 28.6  | 38.8          |
| β-globulins %   |          | 21.7±  | 16.3b  | 2.6   | 16.0  | 12.4  | 20.9  | 16.4± | 3.2   | 15.8  | 12.7  | 23.9  | 13.2  | 21.7          |
| γ-globulins %   |          | 21.2±  | 21.0b  | 3.7   | 20.5  | 16.3  | 27.4  | 23.4± | 5.9   | 23.6  | 8.1   | 35.9  | 17.7  | 30.7          |
| n               | 1        | 10     | 23     |        |       |      |      |      |        |       |      |      |                |

Abbreviations: BCG, Bromocresol green dye-binding method; CZE, capillary zone electrophoresis.

*Not normally distributed.

Normally distributed.
| Parameter          | Unit     | Spring | Fall | Summer | Mean | SD    | Median | Min   | Max   | Mean | SD    | Median | Min   | Max   | 10%   | 90%   | 90%   | 90%   | 90%   |
|--------------------|----------|--------|------|--------|------|-------|--------|-------|-------|------|-------|--------|-------|-------|-------|-------|-------|-------|-------|
| tT4 ng/mL          | 12.54    | 2.9    | 2.6  | 2.9    | 1.24 | 0.28  | 1.24   | 0.84 | 1.62  | 1.24 | 0.28  | 1.24   | 0.84 | 1.62  | 1.24  | 0.28  | 1.24  | 0.84 |
| fT4 pmol/L         | 220.29   | 161.0  | 161.0| 161.0  | 175.06| 4.50  | 175.06 | 175.06| 175.06| 175.06| 4.50  | 175.06 | 175.06| 175.06| 175.06| 4.50  | 175.06| 175.06|
| tT3 ng/mL          | 0.28     | 0.07   | 0.07 | 0.07   | 0.07 | 0.07  | 0.07   | 0.07 | 0.07  | 0.07 | 0.07  | 0.07   | 0.07 | 0.07  | 0.07  | 0.07  | 0.07  | 0.07 |
| fT3 pmol/L         | 1.86     | 1.48   | 1.86 | 1.86   | 1.48 | 1.86  | 1.48   | 1.86 | 1.86  | 1.48 | 1.86  | 1.48   | 1.86 | 1.86  | 1.48  | 1.86  | 1.86  | 1.48 |
| n                  | 10       | 31     | 55   | 55     | 55   | 55    | 55     | 55   | 55    | 55   | 55    | 55     | 55   | 55    | 55    | 55    | 55    | 55   |
| Iodine µg/L        | 148.30b  | 146.72 | 146.72| 146.72 | 146.72| 146.72| 146.72 | 146.72| 146.72| 146.72| 146.72| 146.72 | 146.72| 146.72| 146.72| 146.72| 146.72| 146.72|
| Total protein g/L  | 33.1a    | 10.3   | 10.3 | 10.3   | 10.3 | 10.3  | 10.3   | 10.3 | 10.3  | 10.3 | 10.3  | 10.3   | 10.3 | 10.3  | 10.3  | 10.3  | 10.3  | 10.3 |
| Albumin (BCG)      | 1.86a    | 1.24   | 1.24 | 1.24   | 1.24 | 1.24  | 1.24   | 1.24 | 1.24  | 1.24 | 1.24  | 1.24   | 1.24 | 1.24  | 1.24  | 1.24  | 1.24  | 1.24 |
| Prealbumin(CZE) %  | 11.0b    | 7.0    | 7.0  | 7.0    | 7.0  | 7.0   | 7.0    | 7.0 | 7.0   | 7.0  | 7.0   | 7.0    | 7.0 | 7.0   | 7.0   | 7.0   | 7.0   | 7.0 |
| Albumin (CZE) %    | 28.1b    | 4.5    | 4.5  | 4.5    | 4.5  | 4.5   | 4.5    | 4.5 | 4.5   | 4.5  | 4.5   | 4.5    | 4.5 | 4.5   | 4.5   | 4.5   | 4.5   | 4.5 |
| A:G ratio %        | 0.67a    | 0.22   | 0.22 | 0.22   | 0.22 | 0.22  | 0.22   | 0.22 | 0.22  | 0.22 | 0.22  | 0.22   | 0.22 | 0.22  | 0.22  | 0.22  | 0.22  | 0.22 |
| α1-globulins %     | 27.6b    | 5.5    | 5.5  | 5.5    | 5.5  | 5.5   | 5.5    | 5.5 | 5.5   | 5.5  | 5.5   | 5.5    | 5.5 | 5.5   | 5.5   | 5.5   | 5.5   | 5.5 |
| Albumin (CZE) %    | 28.1b    | 4.5    | 4.5  | 4.5    | 4.5  | 4.5   | 4.5    | 4.5 | 4.5   | 4.5  | 4.5   | 4.5    | 4.5 | 4.5   | 4.5   | 4.5   | 4.5   | 4.5 |
| Albumin (CZE) %    | 28.1b    | 4.5    | 4.5  | 4.5    | 4.5  | 4.5   | 4.5    | 4.5 | 4.5   | 4.5  | 4.5   | 4.5    | 4.5 | 4.5   | 4.5   | 4.5   | 4.5   | 4.5 |
| γ-globulins %      | 34.4     | 34.4   | 34.4 | 34.4   | 34.4 | 34.4  | 34.4   | 34.4 | 34.4  | 34.4 | 34.4  | 34.4   | 34.4 | 34.4  | 34.4  | 34.4  | 34.4  | 34.4 |
| n                  | 10       | 31     | 55   | 55     | 55   | 55    | 55     | 55   | 55    | 55   | 55    | 55     | 55   | 55    | 55    | 55    | 55    | 55   |

Abbreviations: BCG, Bromocresol green dye-binding method; CZE, capillary zone electrophoresis.
| Parameter          | Unit  | Spring          | Summer          | Fall            |
|--------------------|-------|-----------------|-----------------|-----------------|
|                    | Mean  | SD              | Median          | Min  | Max  | Mean  | SD              | Median          | Min  | Max  | Mean  | SD              | Median          | Min  | Max  | Mean  | SD              | Median          | Min  | Max  | Mean  | SD              | Median          | Min  | Max  |
| tT4                | µg/dL | 1.53a           | 0.81            | 1.90 | 1.20 | 1.90b | 2.03            | 1.10 | 0.06 | 6.40            | 0.63a           | 1.23 | 0.06 | 0.06 | 3.40            |
| fT4                | pmol/L| 26.20a          | 16.46           | 20.30 | 13.50 | 44.80 | 29.45b          | 29.14 | 13.5 | 5.20 | 112.30 | 11.13b           | 17.66           | 3.30 | 2.00 | 50.70            |
| tT3                | ng/mL | 0.54a           | 0.22            | 0.44 | 0.39 | 0.79  | 6.31a           | 4.13 | 4.98 | 1.81 | 15.35 | 10.16a           | 3.79            | 11.62 | 3.63 | 13.95            |
| fT3                | pmol/L| 1.33b           | 0.46            | 1.60 | 0.80 | 1.60  | 1.19a           | 0.68 | 1.00 | 0.40 | 2.60  | 0.70a            | 0.70            | 0.70 | 0.0  | 2.10             |
| n                  | 3     | 13              | 7               | 7                | 7                | 7                | 7                | 7                | 7     | 7                | 7                | 7                |
| Iodine             | µg/L  | 23.05a          | 5.44            | 23.05 | 19.20 | 26.9  | 32.09a          | 13.54 | 28.30 | 17.90 | 57.40 | 106.19a          | 76.68           | 135.00 | 18.00 | 230.00          |
| Total protein      | g/L   | 24.0a           | 2.7             | 24.9 | 21.0 | 26.2  | 35.1a           | 14.1 | 33.0 | 14.5 | 53.2  | 44.1a            | 6.6             | 44.3 | 31.9 | 51.5             |
| Albumin (BCG)      | g/L   | 10.2a           | 0.6             | 10.1 | 9.7  | 10.9  | 9.9a            | 6.4  | 9.9  | 2.2  | 20.2  | 18.6a            | 3.5             | 18.7 | 11.8 | 22.8             |
| Albumin total (CZE)| g/L   | 6.2a            | 0.35            | 6.2  | 5.9  | 6.6   | 10.9a           | 5.3  | 9.3  | 4.1  | 22.3  | 16.3*            | 5.3             | 18.4 | 8.3  | 23.3             |
| Albumin total (CZE)| %     | 26.2b           | 4.5             | 23.7 | 23.6 | 31.4  | 30.5a           | 5.8  | 30.5 | 21.5 | 44.4  | 36.3a            | 7.9             | 37.8 | 25.3 | 45.3             |
| Prealbumin (CZE)   | %     | 4.5a            | 1.0             | 5.0  | 3.4  | 5.1   | 6.8b            | 4.3  | 4.6  | 2.3  | 16.8  | 13.1a            | 6.5             | 14.2 | 4.5  | 21.8             |
| Albumin (CZE)      | %     | 21.7a           | 4.1             | 20.2 | 18.6 | 26.3  | 23.7a           | 2.8  | 23.3 | 19.2 | 29.4  | 23.2a            | 2.5             | 23.5 | 20.2 | 26.5             |
| A:G ratio          | %     | 0.36b           | 0.09            | 0.31 | 0.31 | 0.46  | 0.45b           | 0.13 | 0.44 | 0.27 | 0.80  | 0.59a            | 0.19            | 0.61  | 0.34 | 0.83             |
| α-globulins        | %     | 39.1a           | 8.9             | 34.9 | 33.0 | 49.3  | 32.0a           | 5.3  | 31.8 | 22.8 | 40.5  | 32.7a            | 8.1             | 33.1 | 23.3 | 45.8             |
| β-globulins        | %     | 17.1a           | 3.0             | 16.0 | 14.9 | 20.5  | 17.4a           | 3.2  | 17.2 | 12.9 | 23.0  | 14.6a            | 2.1             | 15.2 | 11.0 | 17.1             |
| γ-globulins        | %     | 17.6a           | 5.9             | 18.8 | 11.1 | 22.8  | 20.1a           | 4.9  | 19.5 | 11.5 | 30.0  | 16.5a            | 3.8             | 14.6 | 13.0 | 21.8             |

Abbreviations: BCG, Bromocresol green dye-binding method; CZE, capillary zone electrophoresis.

*a Normally distributed.

*Not normally distributed.
TABLE 4  Thyroid hormone and plasma protein levels using capillary zone electrophoresis in female map turtles (Graptemys spp.) in different seasons

| Parameter | Unit      | Spring          |               | Summer          |               | Fall           |               | 10% Percentile | 90% Percentile |
|-----------|-----------|-----------------|---------------|-----------------|---------------|----------------|---------------|----------------|----------------|
| tT4       | µg/dL     | 1.20±           | 0.46          | 1.10            | 0.80          | 1.70±          | 0.66          | 1.90           | 1.40           | 2.70±          | 0.95           | 0.06           | 3.80           | 0.06           | 2.80           |
| FT4       | pmol/L    | 20.67±          | 5.33          | 22.80           | 14.60         | 24.60±         | 35.73±        | 11.57          | 31.60          | 26.80±         | 48.80±         | 19.71±         | 16.10          | 19.60          | 1.00           | 54.70±         | 2.20           | 43.90           |
| tT3       | ng/mL     | 2.47±           | 2.99          | 1.12            | 0.39          | 5.89±          | 2.41±         | 3.40           | 0.55           | 0.35           | 6.34±          | 5.08±          | 4.70           | 2.17           | 0.50           | 12.44±         | 0.57           | 10.83           |
| FT3       | pmol/L    | 2.33±           | 0.40          | 2.40            | 1.90          | 2.70±          | 1.70±         | 0.62           | 1.50           | 1.20           | 2.40±          | 0.85±          | 0.62           | 0.85           | 0.00           | 2.40±          | 0.10           | 1.40           |
| Iodine    | µg/L      | 56.10±          | 8.15          | 56.40           | 47.80         | 64.10±         | 75.80±        | 66.44          | 59.10          | 19.10          | 149.00±        | 13.81±         | 31.20          | 33.3           | 59.2           | 19.30          | 149.00±        | 32.38±         | 59.10           |
| Albumin   | g/L       | 18.5±           | 6.4           | 16.5            | 13.4          | 25.7±          | 19.2±         | 8.5            | 15.0           | 13.5           | 29.0±          | 16.9±          | 5.5            | 16.4           | 9.1            | 28.8           | 11.3           | 24.1           |
| Albumin   | g/L       | 18.7±           | 2.7           | 19.3            | 15.8          | 21.1±          | 17.8±         | 4.7            | 19.4           | 12.5           | 21.4±          | 15.0±          | 5.3            | 14.9           | 6.3            | 24.9           | 9.1            | 22.9           |
| Albumin   | %         | 49.3±           | 7.1           | 53.4            | 41.1          | 53.4±          | 39.5±         | 4.7            | 37.5           | 36.1           | 44.8±          | 34.8±          | 5.5            | 35.3           | 22.6           | 44.3           | 27.8           | 42.5           |
| Prealbumin| %         | 22.8±           | 5.7           | 22.3            | 17.4          | 28.7±          | 10.3±         | 5.3            | 9.9            | 5.3            | 15.8±          | 10.8±          | 5.6            | 11.2           | 2.5            | 20.3           | 3.9            | 17.6           |
| Albumin   | %         | 26.5±           | 4.0           | 24.7            | 23.7          | 31.1±          | 29.1±         | 1.6            | 29.0           | 27.6           | 30.8±          | 23.9±          | 2.9            | 24.3           | 18.7           | 28.1           | 20.1           | 27.1           |
| A:G ratio | %         | 1.00±           | 0.26          | 1.15            | 0.70          | 1.15±          | 0.66±         | 0.13           | 0.60           | 0.56           | 0.81±          | 0.54±          | 0.13           | 0.55           | 0.29           | 0.80           | 0.39           | 0.74           |
| α-globulins| %        | 23.8±           | 6.7           | 21.8            | 18.3          | 31.3±          | 25.8±         | 8.0            | 21.8           | 20.5           | 35.0±          | 27.2±          | 3.13           | 27.3           | 20.2           | 32.6           | 22.9           | 30.7           |
| β-globulins| %        | 11.7±           | 1.9           | 12.3            | 9.5           | 13.2±          | 14.7±         | 0.5            | 14.6           | 14.3           | 15.3±          | 15.7±          | 3.4            | 15.5           | 9.1            | 22.5           | 12.3           | 19.8           |
| γ-globulins| %        | 15.2±           | 0.1           | 15.3            | 15.1          | 15.3±          | 20.0±         | 6.25           | 19.1           | 14.3           | 26.7±          | 22.3±          | 3.9            | 22.5           | 14.3           | 32.1           | 17.9           | 26.4           |

Abbreviations: BCG, Bromocresol green dye-binding method; CZE, capillary zone electrophoresis.

aNormally distributed.
bNot normally distributed.
a CV of 0.12 for the tT4 test on the IMMULITE, an SD of 11.57 and a CV of 0.22 for the fT4 test, and an SD of 0.15 and a CV of 0.27 for the fT3 test. The comparison of the two test systems for tT4 showed an SD of 0.27, a CV of 0.09, a slope of 0.84, a y-intercept of 0.58, an S of 0.44, and an r of .93. The short-term replication of the A:G ratio using CZE showed an SD of 0.03 and a CV of 0.06, while the long-term replication had an SD of 0.09 and a CV of 0.18.

3.2 | Thyroid hormone levels, iodine, and plasma protein analysis in red-eared sliders

A total of 135 samples were collected from red-eared sliders. Four samples were excluded from the statistical analysis due to a low PCV. One hundred and thirty-one samples were evaluated, 34 from males (Table 1: one in spring, 10 in summer, and 23 in fall) and 97 from females (Table 2: 10 in spring, 31 in summer, and 56 in fall). The results for the single male turtle in spring are included, but comparisons based on these values should be considered limited. The tT4 levels in the males increased from spring to summer and decreased to the lowest levels in fall. In females, the mean tT4 value was highest in the spring and decreased to fall, but the highest individual maximum level was measured in the summer (Table 2). The fT4 levels in both males and females showed the same pattern as the tT4 levels. In males, the fT4 levels increased from spring to summer and decreased in the fall. In females, the fT4 levels were highest in spring and decreased in the fall, but the highest individual maximum levels were found in the summer. The mean tT4 and fT4 levels in females were higher than in males during spring and summer, but lower in the fall. The tT3 levels increased in males and females from spring to fall. The females had higher mean tT3 levels in summer and fall than the males. The fT3 levels in males increased from spring to summer and decreased in the fall, and, in females, the levels were highest in spring and decreased in the fall. Iodine increased in males from spring to summer and decreased in the fall. In female red-eared sliders, the iodine levels were highest in spring and decreased in the fall.

One hundred and thirty-one samples were evaluated by CZE, the results from one female turtle in fall were excluded from further analysis because the electropherogram deviated strongly from those of other turtles (Tables 1 and 2). The TP levels decreased in male turtles from spring to summer and increased in the fall. In females, the total protein increased from spring to fall. Albumin levels detected using the BCG method showed a similar progression; albumin increased in males from spring to summer and increased in the fall and increased in females from spring to fall. The total albumin levels measured by CZE had a contrary progression in that the levels decreased in males continuously from spring to fall and increased in females from spring to summer and increased in the fall. The albumin-globulin (A:G) ratio using CZE increased in males from spring to summer and decreased in the fall. In females, the ratio decreased from spring to summer and increased in the fall. The α-globulin fraction increased in males from spring to summer and decreased slightly in the fall. In females, α-globulin levels developed differently, increasing from spring to summer and decreasing in the fall. In males, β-globulins decreased from spring to summer and increased slightly in the fall. In females, β-globulins increased from spring to summer and decreased in the fall. The γ-globulin fraction decreased slightly in males from spring to summer and increased in the fall. In females, γ-globulins increased continuously from spring to fall.

3.3 | Thyroid hormone levels, iodine, and plasma protein analyses in map turtles

Turtles in the genus Graptemys were not further divided into the different species in the present study because of the low number of turtles of different species and the difficulty inherent in differentiating between specific species and hybrids. A total of 52 plasma samples were collected from map turtles, one was excluded from the statistical analysis due to a low PCV. Fifty-one samples were evaluated, 23 from males (Table 3: three in spring, 13 in summer, and seven in fall) and 28 from females (Table 4: three in spring, three in summer, and 22 in fall). Only a lower number of serum samples were available for measuring iodine in male map turtles because lithium heparinized samples were taken first, and the collected blood volume was limited because not enough blood was obtained for all tests in all cases (Table 3). The tT4 and fT4 levels in both sexes increased from spring to summer and decreased in the fall, in contrast to the levels in female red-eared sliders. The males had higher tT4 and fT4 levels in spring but lower levels in summer and fall compared with the levels in females. In comparison with the red-eared sliders, the map turtles had lower tT4 and fT4 levels. In males, the tT3 levels increased from spring to fall, like the levels in red-eared sliders. In females, the tT3 levels decreased slightly from spring to summer and increased in the fall. The tT3 levels in males developed in inverse proportions to the tT3 levels. The fT3 levels in both sexes decreased from spring to fall, like the levels in red-eared sliders. The iodine levels in males were lowest in spring and increased in the fall. In females, the iodine levels increased from spring to summer and decreased in the fall, similar to the levels in male red-eared sliders.

The results of the protein analyses are based on the same number of samples as the thyroid hormone levels (Tables 3 and 4). The TP levels increased continually in males from spring to fall and increased in females from spring to summer but decreased in the fall. Albumin measured using the BCG method decreased from spring to summer and increased in the fall. In females, albumin increased in the summer and decreased in the fall. Total albumin measured using CZE increased continually in males and decreased continually in females from spring to fall. The A:G ratio measure using CZE increased in males and decreased in females from spring to fall. The α-globulin fraction decreased in males from spring to summer and increased
slightly in the fall. In females, the α-globulin fraction increased continually from spring to fall. In males, the β- and γ-globulins increased in the summer and decreased in the fall. In females, the levels increased continually from spring to fall.

The statistical evaluation of differences between the thyroid hormones and iodine between the species, sexes, and seasons are shown in Tables 5-7.

3.4 | Statistical evaluation of the plasma protein fractions and correlation with thyroid hormones

Correlations between protein levels according to species, sex, and season are shown in Table 5. The total albumin levels measured using the BCG and CZE methods showed highly significant differences in male red-eared sliders in the fall (P < .0001) and significant differences in male map turtles in the spring (P = .0005). Correlations between TP and total albumin concentrations measured with CZE and the thyroid hormone levels in red-eared sliders and map turtles are shown in Tables 6 and 7, respectively.

### TABLE 5 Significant variations in thyroid hormones, iodine, and plasma proteins between the species, sexes, and seasons

| Parameter                  | Significant variations between                                                                 |
|----------------------------|-----------------------------------------------------------------------------------------------|
| tT4                        | species (P < .0001), seasons (P < .0001)                                                        |
| FT4                        | species (P < .0001), seasons (P < .0001)                                                        |
| tT3                        | species (P = .0235), seasons (P = .0002).                                                      |
| FT3                        | seasons (P = .0011)                                                                            |
| Iodine                     | species (P = .0238), in combination of species and season (P = .0067), and in combination of sex and season (P = .0061) |
| Total protein              | seasons (P = .0003)                                                                            |
| Albumin (BCG)              | season (P < .0001), in combinations of season and sex (P = .0062)                             |
| Albumin total (CZE g/L)    | sexes (P = .0111), the season (P = .0077), and in a combination of season and sex (P = .0376) |
| Albumin (total CZE %)      | species (P = .0027), sexes (P < .0001), in combination of sex and season (P = .0091), and in combination of species, sex and season (P = .0328) |
| Prealbumin (CZE %)         | species (P = .0254), sexes (P = .0028), in combination of species, sex and season (P = .0078) |
| Albumin (CZE %)            | sex (P = .0126)                                                                               |
| A:G ratio                  | sexes (P < .0001), species (P = .0015), in combination of season and sex (P = .0030), and in combination of species, season and sex (P = .0185) |
| α-globulins                | sexes (P < .0001), and in combination of species and sex (P = .0196)                          |
| β-globulins                | sexes (P = .0240), and in combination of sex and season (P = .0482)                           |
| γ-globulins                | None                                                                                         |

4 | DISCUSSION

The results of the present study showed that not all thyroid hormones were correlated with TP and albumin levels in regard to either sex or season. This is in contrast to the results of previous studies, in which the binding proteins (TBP and albumin) and T4 levels were highly correlated. Licht et al reported that TBP in turtles (T scripta) was near the albumin fraction on polyacrylamide-gel electrophoresis, which correlated with the α1-fraction in the present study. However, it is important to note that TBP was not specifically determined in the present study. The albumin levels, as measured with CZE, were used for the evaluation of the correlations between the thyroid hormone levels in this study. Previous studies demonstrated that albumin levels in tortoises measured using the BCG method were higher than when they were measured with cellulose acetate electrophoresis (CAE). The authors concluded that electrophoresis is preferable for the correct measurement of albumin. A study comparing albumin levels measured using CZE with those measured using the BCG method in Hermann’s tortoises (Testudo hermanni) showed that the differences between the two detection methods varied depending on sex and season. Similar results were obtained for the comparisons in the present study. The albumin levels measured using the BCG method were higher in male red-eared sliders and map turtles in spring and fall and in female red-eared sliders and map turtles in summer and fall. There are several possible explanations for these differences; it is possible that substances other than albumin, such as the α-globulin fraction proteins, could bind to the reagent of the BCG test, especially if the test reaction time is too long. To exclude this factor, the reaction time for the BCG test was standardized according to the guidelines of the reagent manufacturer and the European Federation of Clinical Chemistry and Laboratory Medicine. Another possible explanation could be that the human standard reagent used in the BCG test has a different binding affinity to chelonian albumin. There is, however, no specific standard reagent for chelonian albumin available.

Considering these differences and variations, it is most appropriate to measure albumin levels in turtles using electrophoresis, and the BCG test is not recommended in these animals.

Interestingly, CZE led to the differentiation of more peaks in both the sliders and map turtles than described in Hermann’s tortoises using the same technology (Figures 1 and 2). In red-eared sliders (Figure 1) and map turtles (Figure 2), it was possible to differentiate prealbumin, split albumin, split α-globulin, split β-globulin, and single γ-globulin peak. To allow for comparisons with previous studies in red-eared sliders, and between the two detection methods for albumin, the fractions of the electropherograms were divided into a total albumin fraction that included both prealbumin and albumin, as well as the α, β-, and γ-globulin fractions. Fractions were defined based on the graphic evaluation of the electropherograms, beginning with a single γ-globulin peak on the right because this fraction is similar in all healthy chelonian species using a variety of different techniques. The separation of the other globulin fractions, albumin and prealbumin fractions differed more between species and
TABLE 6 Significant correlations between the different thyroid hormones and the thyroid hormones with the blood iodine and protein levels in red-eared sliders (Trachemys scripta elegans)

| Parameter          | tT4     | fT4     | tT3     | fT3     |
|--------------------|---------|---------|---------|---------|
| fT4                | Positive in males in summer (P = .0029) and fall (P < .0001); and females in all seasons (P < .0001) | None | None | None |
| tT3                | Negative in males (P = .0131), and females (P < .0001) in fall | Negative in males (P = .0083); and females (P < .0001) in fall | None | Negative in females in summer (P = .0175) and fall (P = .0093) |
| fT3                | Positive in females (P = .0015) in fall | Positive in females in fall (P = .0028) | None | None |
| Iodine             | Positive in females in spring (P = .0020), summer and fall (P < .0001); and males (P < .0001) in fall | Positive in females in spring (P < .0001), summer (P = .0024), and fall (P < .0001); and in males (P < .0001) in fall | Negative in males (P = .0070); and females (P < .0001) in fall | None |
| Total protein      | Positive in females (P = .0130) in fall | Positive in females (P = .0225) in fall | Negative in females (P = .0395) in fall | None |
| Albumin total (CZE g/L) | Positive in females (P = .0089) in fall | Positive in females (P = .0195) in fall | Negative in females (P = .0053) and males (P = .0474) in fall | None |

TABLE 7 Significant correlations between different thyroid hormone levels and between thyroid hormone levels and blood iodine and protein levels in map turtles (Graptemys spp.)

| Parameter          | tT4     | fT4     | tT3     | fT3     |
|--------------------|---------|---------|---------|---------|
| fT4                | Positive in males in summer (P < .0001); and females (P < .0001) in all seasons | None | Positive in males (P < .001) in spring; Negative in females (P = .0459) in fall | None |
| tT3                | Negative in females (P = .0388) in fall | None | None | None |
| fT3                | Positive in females (P = .0150) in fall | Positive in females (P = .0119) in fall | None | None |
| Iodine             | Positive in females (P < .0001) in summer; Negative (P = .0399) in males in fall | Positive in females in summer (P < .0001), and fall (P = .0405) | None | Positive in females (P < .0001) in spring |
| Total protein      | Negative in males (P < .0001) in summer; Positive in females in summer (P < .0001), and fall (P = .0094) | Positive in females in summer (P < .0001), and fall (P = .0071) | Positive in females in spring (P < .0001), and fall (P = .0108) | None |
| Albumin total (CZE g/L) | Positive in females in summer (P < .0001), and fall (P = .0252) | Positive in females in summer (P < .0001), and fall (P = .0182) | Positive in females (P < .0001) in spring, negative in females (P = .0076) in fall | None |

electrophoretic techniques based on the separation principle and the different protein structures. The fractions found in the turtle species studied are comparable to those found in Hermann’s tortoises using CZE. 23 The main differences between the tortoises and turtles in the present study are the split albumin fractions in most of the turtles examined. A split albumin peak has also been described in green iguanas (Iguana iguana) and could be genetic in origin. 26 Comparisons between the present CZE results and previous studies on emydid turtles show that CZE found split albumin and β-globulin peaks in contrast to agarose gel electrophoresis which was able to differentiate prealbumin, albumin, α1-, α2-, β-, and γ-globulin peaks in free-ranging eastern box turtles (Terrapene carolina carolina). 27 Agarose gel electrophoresis and CAE were both able to differentiate albumin, α-, β-, and γ-globulin peaks in red-eared sliders in May and June. 26 In eastern box turtles, TP and albumin levels were the highest in summer, 27 while the highest albumin peak was found in the fall in red-eared sliders and male map turtles in the present study. The A:G ratio in box turtles did not significantly vary between the seasons, 27 similar to the results obtained in the present study. Significantly higher TP levels in females, as described by Flower et al, 27 were not found in the present study.

Giménez et al 26 have shown that hemolysis affects the α1- and β-fractions in red-eared sliders. To eliminate such interferences and
changes in TP, in the present study, only samples that showed no visible abnormalities in color, transparency, or consistency were included. The results of Martínez-Subiela et al.\textsuperscript{28} and Bossuyt et al.\textsuperscript{29} indicated that fibrinogen does not cause an increase in the β-globulin fraction when using CZE, which demonstrated that it is possible to compare serum and plasma results directly. This is important because reptile blood often coagulates relatively slowly,\textsuperscript{30} making the analysis of serum impossible. Lithium heparin plasma is, therefore, preferable for laboratory testing.

Moore et al.\textsuperscript{31} reported that a ranavirus infection led to significant changes in protein levels in red-eared sliders that changed over the course of the infection. This indicates that electrophoresis might be helpful for the diagnosis and assessment of infectious diseases in turtles.

In canine and feline medicine, tT4 measurements are often used as an initial screening test for thyroid function. fT4 is used as a more sensitive and specific indicator, and thyroid-stimulating hormone (TSH) is used for further diagnostics.\textsuperscript{32} The results of the present study showed that the thyroid hormone levels in turtles are influenced by the season, which is different from most mammals and shows the complexity of these hormones in turtles. In birds and reptiles, tT4 has also been used as an initial screening test.\textsuperscript{33,34}

Currently, most published studies of thyroid function in chelonians are based on tT4 measurements.\textsuperscript{4,7-9} No studies are available that measure all thyroid hormones (tT4, fT4, T3, fT3) as well as iodine in chelonians, especially in turtles. Bühler\textsuperscript{3} measured tT4, fT4, fT3, and fT3 in several chelonian species and showed low thyroid hormone levels in tortoises. The tT3 levels of desert tortoises (Gopherus agassizii) reported by Kohel et al.\textsuperscript{5} were under the detection limit of the test used. In the present study, strong variations were noted in the tT4 and fT4 levels between the different seasons. In summer, some turtles had levels above the detection limit of the canine tT4 test (>15 µg/dL), while in the fall, some turtles had levels below the detection limit (<0.12 µg/dL) of this test. To obtain the exact values for the turtles with high hormone levels, the samples were retested with a human tT4 test that had a higher detection limit (>24 µg/dL), and those samples that continued to have higher levels were diluted and retested. The same problem was encountered with fT4 measurements. Samples with levels above the detection limit of 100 pmol/L were also diluted and retested. The significant variations in all of the thyroid hormone levels, in the present study, makes it impossible to find a single, adequate test capable of detecting both the very high levels in summer and the low levels in fall.

Sawin et al.\textsuperscript{35} showed that tT4 increased after an injection with ovine TSH in Chrysemys picta, which indicates that reptile TSH has an analogous structure and function to that of mammals. Currently, however, there is no commercial test available that can detect TSH levels in reptile blood. TSH was, therefore, not included in the present study. In comparison to the tT4 levels reported in tortoises,\textsuperscript{3-6} the tT4 levels in turtles, especially in red-eared sliders, were very high in summer. Labrada-Martagón et al.\textsuperscript{8} also reported lower tT4 levels in green sea turtles compared with the high levels in summer, in the present study. The fT4 levels in tortoises\textsuperscript{6} were distinctly lower than those in the aquatic turtles, in the present study. There were, however, no clear differences between the tT3 and fT3 levels measured in tortoises\textsuperscript{6} and those measured in the turtles, in the present study.

Significant differences were documented between tT4, fT4, and T3 levels in the red-eared sliders and map turtles (Tables 1-4). The analogous progression of the tT4 and fT4 levels in the different seasons, and the calculated correlations, show that the levels influence each other in both sexes and species over the course of the year. This correlates with the temperature and light cycle in Germany and shows that tT4 and fT4, like the metabolic rate, are influenced by external temperatures. Although the red-eared sliders and map turtles were all housed in greenhouses under comparable conditions and fed the same diet, the red-eared sliders still had higher iodine levels, correlating with the higher tT4 and fT4 levels found in this species. The tT3 and fT3 levels rarely correlated with tT4 or, in some cases, correlated negatively, such as with tT3 in male and female red-eared sliders in fall. Studies in dogs have shown breed-specific influences on the tT4 levels,\textsuperscript{32} and in Amazon parrots, tT4 levels also vary
depending on the species. Studies in dogs and cats also show that age influences thyroid hormone levels. A similar effect has also been documented in desert tortoises in July, with higher levels found in adult males compared with those in juveniles and subadult tortoises. All of the turtles included in the present study were adults, and no age differences were documented.

There are numerous factors that can influence thyroid hormone production. Labrada-Martagón et al. showed a positive correlation between blood glucose levels and tT4 levels in immature sea turtles (Chelonia mydas), which could be an indication that food intake influences thyroid hormone levels. However, the foods fed to the green sea turtles in that study (shrimp, snails, ctenophores, and seagrasses) are also high in iodine. Increased dietary iodine could, therefore, be responsible for higher iodine blood levels that, in turn, increase thyroid hormone levels. Because iodine is an essential mineral for thyroid hormone synthesis, a deficiency leads to a decrease in thyroid hormone levels associated with hyperplasia and hypertrophy of thyroid follicular cells. Boogs et al. showed a significant correlation between plasma iodine concentrations and tT4 concentrations in juvenile American alligators (Alligator mississippiensis) from a freshwater habitat in Florida. However, no significant correlations were found between tT4 or tT3 concentrations and iodine levels in alligators from an estuarine habitat. In the red-eared sliders and map turtles of this study, a significant correlation between iodine and tT4 and tT4 was found for some sexes in some seasons. For instance, tT3 correlated negatively with iodine in the male and female red-eared sliders only in the fall, while tT3 correlated positively with iodine in female map turtles only in spring. The iodine levels did not differ significantly for sex and time of the year in American alligators, which was in contrast with the results for the present study, where significant variations were found for combinations of species and season and sex and season. Iodine concentrations were the highest in seawater and decreased from sea to inland depending on the rainfall amounts and soil types. Germany, in general, is considered an iodine-deficient area, which likely affected the iodine concentrations available to the turtles through the environment and diet, for which a large part consisted of lettuce, wild herbs, and water plants from southern Germany. However, the iodine concentrations in the turtle blood were relatively high. In the red-eared sliders, in the summer, iodine levels were even higher than those published for American alligators.

The present results document the strong variations in the thyroid hormone levels and plasma protein fractions between different aquatic turtle species and during different times of the year. Both tT4 and FT4 were highest during the most active phase in the summer. Further studies are necessary to evaluate whether high tT4 and FT4 levels are a cause of the higher activity of the turtles or if these factors are independent of each other. The results of this study form a basis for further investigations to recognize the effects of thyroid hormone variations on turtle metabolism. The presented fluctuating plasma protein levels are also important for understanding turtle metabolism and could be helpful for monitoring specific organ functions, such as those of the liver or immune system, to evaluate the effects of specific disease processes. Only suitable comparative values can enable early diagnosis of thyroid diseases and protein imbalances and can allow for follow-up studies during the medical treatment in these cases.

ACKNOWLEDGMENTS

This study was partially funded by a research and conservation grant from the Association of Reptilian and Amphibian Veterinarians (ARAV), a grant from the Pauler Fund of the AG ARK of the “Deutsche Gesellschaft für Herpetologie und Terrarienkunde (DGHT),” and by a Wild Animal Health Fund grant from the American Association of Zoo Veterinarians (AAZV). The authors thank Dr. Karl Rohn of the Department of Biometry, Epidemiology, and Information Processing of the University of Veterinary Medicine Hannover for help with the statistical analysis of the results. We also thank Dr. Florian Brandes from the wildlife conservation center in Sachsenhagen and the team of the reptile rescue center in Munich for their help with the collection of blood samples.

ORCID

Christoph Leineweber https://orcid.org/0000-0003-4241-7018

REFERENCES

1. Rivera S, Lock B. The reptilian thyroid and parathyroid glands. Vet Clin North Am Exot Anim Pract. 2008;11:163-175.
2. Licht P, Denver RJ, Pavgi S. Temperature dependence of in vitro pituitary, testis and thyroid secretion in a turtle, Pseudemys scripta. Gen Comp Endocrinol. 1989;80:238-256.
3. Bühler I. Schildrüsenparameter bei häufig in der tierärztlichen Praxis vorgestellten Landschildkrötenarten (Thyroid parameters in tortoises and turtles frequently presented at the veterinary practise). [Dissertation]. Ludwig Maximilian University Munich, Germany; 2006.
4. Franco KH, Famini DJ, Hoover JP, Payton ME. Serum thyroid values for African spurred tortoises (Centrochelys [formerly Geochelone] sulcata). J Herpetol Med Surg. 2009;19:47-49.
5. Kohel KA, Mackenzie DS, Rostal DC, Grumbels JS, Lance VA. Seasonality in plasma thyroxine in the desert tortoise Gopherus agassizii. Gen Comp Endocrinol. 2001:121:214-222.
6. Leineweber C, Öfner S, Mathes K, Marschag RE, Stöhrl AC. Thyroid hormone levels in tortoise (Testudo spp.) depending on season and sex. Vet Clin Pathol. 2013;42:212-216.
7. Norton TM, Jacobson ER, Caliguri R, Kollia G. Medical management of a Galapagos tortoise (Geochelone elephantopus) with hypothyroidism. J Zoo Wildl Med. 1989:20:212-216.
8. Labrada-Martagón V, Méndez-Rodríguez LC, Mangel M, Zenteno-Sávin T. Applying generalized linear models as an explanatory tool of sex steroids, thyroid hormones and their relationships with environmental and physiologic factors in immature East Pacific green sea turtles (Chelonia mydas). Comp Biochem Physiol A Mol Integr Physiol. 2013:166:91-100.
9. Licht P, Breitenbach GL, Congdon JD. Seasonal cycles in testicular activity, gonadotropin and thyroxin in the painted turtle, Chrysemys picta, under natural conditions. Gen Comp Endocrinol. 1985;59:130-139.
10. Licht P, Denver RJ, Herrera B. Comparative survey of blood thyroxine binding proteins in turtle. J Exp Zool. 1991:259:43-52.
11. Pavgi S, Licht P. Measurement of plasma thyroxine binding protein in relation to thyroid condition in the turtle (Trachemys scripta) by radioimmunoassay. Gen Comp Endocrinol. 1992:85:147-155.
12. Licht P, Denver RJ, Stamper DL. 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.
13. Glennemeier KA, Licht P. Binding affinities of thyroxine-binding proteins in turtle plasma. Gen Comp Endocrinol. 1993;90:78-86.
14. Gal J, Csikö G, Pásztor I, Bölcskey-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.
15. Regulation (EU) no 1143/2014 of the European Parliament and of the council of 22 October 2014 on the prevention and management of the introduction and spread of invasive alien species. Available at: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32014R1143&from=DE. Accessed February 11, 2019.
16. Sheldon JD, Stacy NL, Blake S, Cabrera F, Deem SL. Comparison of total leucocyte quantification methods in free-living Galapagos tortoises (Chelonoidis spp.). J Zoo Wildl Med. 2016;47:196-205.
17. Klaphake E, Gibbons PM, Sladky KK, Carpenter JW. Reptiles. In: Carpenter JW, ed. Exotic animal formulary. 5th ed. St. Louise, MO: Elsevier; 2018:134-135.
18. Michalke B, Witte H. Characterization of a rapid and reliable method for iodide biomonitoring in serum and urine based on ion chromatography-ICP-mass spectrometry. J Trace Elem Med Biol. 2015;29:63-68.
19. Friedrichs KR, Harr KE, Freeman KP, et al. ASVCP reference interval guidelines: determination of de novo reference intervals in veterinary species and other related topics. Vet Clin Pathol. 2012;41:441-453.
20. Licht P, Denver RJ, Pavgi S, Herrera B. Seasonality in plasma thyroxine binding in turtles. J Exp Zool. 1991;260:59-65.
21. Macrelli R, Ceccarelli MM, Fiorucci L. Determination of serum albumin concentration in healthy and diseased Hermann’s tortoises (Testudo hermanni): a comparison using electrophoresis and the bromocresol green dye-binding method. J Herpetol Med Surg. 2013;23:20-24.
22. Müller K, Brunnberg L. Determination of plasma albumin concentration in healthy and diseased turtles: a comparison of protein electrophoresis and the bromocresol green dye-binding method. Vet Clin Pathol. 2010;39:79-82.
23. Leineweber C, Stöhr AC, Öfner S, Mathes K, Marschang RE. Reference intervals for plasma capillary zone electrophoresis in Hermann's tortoises (Testudo hermanni) depending on season and sex. J Zoo Wildl Med. 2019;50:611-618.
24. Spagnolo V, Crippa V, Marzia A, Sartorelli P. Reference intervals for hematologic and biochemical constituents and protein electrophoretic fractions in captive common buzzards (Buto buteo). Vet Clin Pathol. 2006;35:82-87.
25. Barber BJ, Stanhope VL. Bromocresol green assay is nonspecific for rat plasma albumin. Am J Physiol. 1992;262(1 Pt 2):H299-H302.
26. Giménez M, Saco Y, Pato R, Busquets A, Martorell JM, Bassols A. Plasma protein electrophoresis of Trachemys scripta and Iguana iguana. Vet Clin Pathol. 2010;39:227-235.
27. Flower JE, Byrd J, Cray C, Allender MC. Plasma electrophoretic profiles and hemoglobin binding protein reference intervals in the eastern box turtle (Terrapene carolina carolina) and influences of age, sex, season, and location. J Zoo Wildl Med. 2014;45:836-842.
28. Martínez-Subiela S, Tecles F, Montes A, Gutiérrez C, Ceron JJ. Effects of haemolysis, lipaemia, bilirubinaemia and fibrinogen on protein electropherogram of canine samples analysed by capillary zone electrophoresis. Vet J. 2002;164:261-268.
29. Bossuyt X, Schiettekatte G, Bogaerts A, Blankaert N. Serum protein electrophoresis by CZE 2000 clinical capillary electrophoresis system. Clin Chem. 1998;44:749-759.
30. Bolten AB, Jacobson ER, Bjorndal KA. Effects of anticoagulant and autoanalyzer on blood biochemical values of loggerhead sea turtles (Caretta caretta). Am J Vet Res. 1992;53:2224-2227.
31. Moore AR, Allender MC, MacNeil AL. Effects of ranavirus infection of red-eared sliders (Trachemys scripta elegans) on plasma proteins. J Zoo Wildl Med. 2014;45:298-305.
32. Scott-Moncrieff JC. Thyroid disorders in the geriatric veterinary patient. Vet Clin North Am Small Anim Pract. 2012;42:707-725.
33. Greenacre CB, Young DW, Behrend EN, Wilson GH. 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.
34. Hernandez-Divers SJ, Knott CD, 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.
35. Sawin TC, Bacharach P, Vance L. Thyrotropin-releasing hormone and thyrotropin in control of thyroid function in the turtle, Chrysemys picta. Gen Comp Endocrinol. 1981;45:7-11.
36. Anderson ET, Minter LJ, Clarke 3rd EO, Mroch 3rd RM, Beasley JF, Harms CA. The effects of feeding on hematological and plasma biochemical profiles in green (Chelonia mydas) and Kemp's Ridley (Lepidochelys kempii) sea turtles. Vet Med Int. 2011;2011:890829.
37. Bogg ASP, Hamlin HJ, Lowers RH, Guillette LJ Jr. Seasonal variation in plasma thyroid hormone concentrations in coastal versus inland populations of juvenile American alligators (Alligator mississippiensis): Influence of plasma iodide concentrations. Gen Comp Endocrinol. 2011;174:362-369.
38. Fuge R, Johnson CC. Iodine and human health, the role of environmental geochemistry and diet, a review. Applied Geochem. 2015;63:282-302.