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Vitamin D_3 in Captive Green Sea Turtles (*Chelonia mydas*)

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Abstract. – **During an accreditation review of the Shark Reef Aquarium at the Mandalay Bay Hotel in Las Vegas, Nevada, AZA (Association of Zoos and Aquariums) reviewers expressed concern about the possible effects of low light intensity and absence of ultraviolet light on serum 25-hydroxyvitamin D levels in our green turtles (*Chelonia mydas*). In an effort to address this concern, blood samples from the aquarium's 3 resident green turtles were periodically analyzed over a 5-year period. Serum 25-hydroxyvitamin D level in a green turtle living at a second indoor facility for 432 months was also analyzed. Two of 4 turtles housed at a third facility were moved from an outdoor to an indoor habitat, and serum 25-hydroxyvitamin D level of all 4 turtles was measured over a 2-year period. In this limited population, serum 25-hydroxyvitamin D levels began to decline about 4–5 months following indoor confinement and continued to decline for 6–8 years. Turtles in the outdoor facility had vitamin D_3 blood serum concentrations of 60–70 nmol/L. After 6–8 years of confinement indoors, blood serum concentrations of vitamin D_3 declined to 5–15 nmol/L. Although clinical symptoms of low serum 25-hydroxyvitamin D levels were not detected during regular veterinary examination of this limited study population, further research is needed to elucidate the long-term effects of restricted ultraviolet exposure or low dietary intake of vitamin D_3 in green turtles. Blood serum concentration of vitamin D_3 in wild turtles has apparently not been reported.**

Key Words. – **Reptilia; Testudines; Cheloniidae; *Chelonia mydas*; sea turtle; vitamin D_3; ultraviolet light; public indoor aquaria; diet**

Mammals synthesize vitamin D_3 in their epidermis, depending on ultraviolet B (UVB) from sunlight to accomplish critical portions of the synthesis. For humans, the process is well enough understood to permit Holick and Jenkins (2003) to develop a guide to optimal levels of sun exposure at various latitudes for individuals that vary over the full range of possible skin color. Optimal levels of vitamin D intake from food are less well understood. For humans, the Food and Drug Administration recommends an intake of 200–400 IU per day, though Holick and Jenkins (2003) suggest that 1000 IU per day would be a more suitable standard. Optimal level of sun exposure or of serum concentration of vitamin D_3 for other vertebrates is far less well understood; although, Holick (2003) notes that most plants and animals exposed to sunlight have the capacity to synthesize vitamin D. Vitamin D is essential for maintenance of calcium homeostasis and therefore critically important for development, growth, and maintenance of a healthy skeleton in vertebrates.

Until recently it was assumed that reptiles, like mammals, synthesize vitamin D_3 only in their epidermis. The assumption has been confirmed in 2 species of tropical anoles (*Anolis* sp.; Ferguson et al. 2005), the house gecko (*Hemidactylus frenatus*) and the Texas spiny lizard (*Sceloporus olivaceus*; Carman et al. 2000), and the corn snake (*Elaphe guttata*; Acierno et al. 2008). Ferguson et al. (2005) further note that skin sensitivity to UVB in these lizard species appears to be inversely correlated to their characteristic natural exposure to sunlight. Nocturnal/crepuscular species or species that spend most of their time in the shade photosynthesize more vitamin D_3 at lower light levels than do species spending more time exposed to direct sunlight. Ramer et al. (2005) have determined that normal serum level of 25-hydroxyvitamin D (25-OH-D) for the wild, healthy Ricord's iguana (*Cyclura ricordii*) and the Rhinoceros iguana (*Cyclura cornuta*) is about 325 nmol/L (130 ng/mL).

Time typically spent exposed to direct sunlight varies substantially between families, genera, and species of turtles and tortoises. This implies major differences in skin sensitivity to vitamin D_3 synthesis and/or differences in dependence on food vs. sunlight exposure to satisfy needs for vitamin D. Chelydrids (snapping turtles) Kinosternids (musk and mud turtles), and perhaps certain other turtle families seem to be able to obtain sufficient vitamin D from their food (they are largely carnivorous). Other families of turtles (notably the freshwater pond turtles, Emydiidae and Bataguriidae), on the other hand, are omnivores and persistent baskers and spend considerable time in direct sunlight, normally perching on rocks or partially sunken logs. Acierno et al. (2006) demonstrate significantly higher plasma concentrations of 25-OH-D in red-eared slider turtles (*Trachemys scripta elegans*) that have had access to basking surfaces illuminated by coil florescent bulbs for 30 days than in turtles held under identical conditions but without the UVB radiation. They further suggest that provision of opportunities to bask in sunlight or under appropriate artificial UVB radiation may be essential to optimal husbandry of red-eared slider turtles. Manning and Grigg (1997) demonstrated that basking of the Australian freshwater side-necked turtle, *Emydura signata*, at the water surface was of no thermoregulatory significance, implying that it may play an important role in the synthesis of 25-OH-D.

Sea turtles (family Cheloniidae) are known to bask out of water only occasionally; although, populations of green turtles (*Chelonia mydas*) that inhabit regions remote from both humans and terrestrial predators such as the French Frigate Shoals in the Hawaiian Islands, the Galapagos Islands, and certain Australian beaches are known to be consistent baskers on shore (Spotila 2004). Whereas green turtles are frequently seen throughout the world floating at the water surface, the effect of this behavior on serum levels of 25-OH-D is unknown; although, it can probably be assumed that some is being synthesized.

During review of the Shark Reef Aquarium for AZA (Association of Zoos and Aquariums) accreditation, reviewers expressed concern about the low light intensity and lack of basking opportunities for green turtles on display. As with many marine aquaria, the Shark Reef Aquarium is an indoor facility where none of the animals on display have access to direct sunlight. AZA reviewers questioned whether such conditions might lead to abnormal or unhealthy conditions, particularly with respect to normal blood levels of vitamin D_3 (25-OH-D) and Ca. On examination of the literature, we could find no information indicating what would be considered ''normal'' levels of vitamin D_3 in either captive or wild green turtles. However, Hasbun et al. (1998) report data on several blood parameters, including Ca in wild sea turtles living near the coastline of the sultanate of Oman in the waters of the Arabian Gulf near the Strait of Hormuz. Similar information is available for a population of juvenile green turtles living in the southern Bahamas (Bolten and Bjorndal 1992). We therefore decided to examine concentrations and variations of vitamin D_3 and other blood parameters in the green turtles held at Shark Reef. We also sought opportunities to obtain information regarding vitamin D_3 levels in the blood of captive green turtles held at other facilities. This study therefore seeks to determine whether differences exist in concentration of vitamin D_3 in the blood of green turtles held indoors compared to those maintained outdoors. The Shark Reef Aquarium maintains 2 adult male light-phase green turtles and 1 juvenile dark-phase green turtle (also known as the east Pacific green turtle or black turtle, *Chelonia mydas agassizii*). To date we have also obtained information about 25-OH-D levels in the blood of 2 green turtles held indoors and 2 held outdoors at the Aquarium of the Pacific, Long Beach, California, and 1 held indoors at the New England Aquarium, Boston, Massachusetts.

METHODS

Two mature male green turtles acquired on 16 June 2000 and 1 immature female acquired on 10 May 2002 from outdoor facilities were, on arrival at the Shark Reef Aquarium, placed in our indoor facility. Beginning on 7 January 2003, all 3 turtles were confined to an isolation pool measuring 15 feet 11 inches × 11 feet 3 inches × 4 feet 9 inches for an average of 2 hours per day, 6 days per week, where they were fed. The isolation pool was illuminated by the same metal halide lighting used throughout the facility in addition to 3 160-W UVB heat lamps (T-Rex Active UVHeat) placed 36 inches above the water surface. UVB intensity measured with a Solartech Digital Ultraviolet Radiometer (model 6.2-UVB) at the water surface directly under new lightbulbs was 100 μW/cm^2 ± 50 μW/cm^2. The heat lamps were replaced every 6 months. Animals were allowed to move freely throughout the enclosed space and did not congregate or ''bask'' directly under the UVB lights. Food was exposed to the UVB lighting until consumed by the turtles, normally an interval of 1–3 minutes.

Elsewhere in the facility where turtles spent the remainder of their day, there was no UVB lighting, and light intensity from programmed metal halide lighting varied from 0 to 4530 lux, was higher during PM than AM hours, and had an average daily variation of 0–2272 lux. Light intensity was measured 55 inches below bulb level at 4 selected locations throughout the facility using a Sper Scientific Broad Range LUX/FC meter model #840022. One location selected was directly below a metal halide bulb, while the others were selected to represent decreasing light intensity as a function of lateral distance from the nearest bulb. Variations in light intensity therefore result from spacing the lights over a large exhibit area and programming them to simulate daily variations in sunlight intensity.

The 3 green turtles maintained at the Shark Reef Aquarium were fed a varied, balanced diet of fresh vegetables (broccoli, Romaine lettuce, green peppers) supplemented with seafoods (squid, prawn, capelin, with occasional substitutions of sardines, herring, and jellyfish) and a weekly vitamin supplement (Sea Tab Marine Animal Vitamins; 3 tabs for the largest male and 2 tabs for the 2 smaller turtles).

Approximately equal quantities of food were ingested by each individual. The smaller, juvenile female was still growing and therefore required proportionately more food than the 2 larger mature males. Food quantity for the 2 males was managed to avoid fat accumulation while maintaining relatively constant weights. Quantity of food

Table 1. Vitamin D_3 (25-OH-D) concentration (nmol/L) in the blood of green turtles held at the Shark Reef Aquarium, Mandalay Bay Hotel, Las Vegas, Nevada (171, 172, 509); the Aquarium of the Pacific, Long Beach, California (Raphael, Michelangelo, Donatello, Leo); and the New England Aquarium, Boston, Massachusetts (Myrtle). Turtles at the Shark Reef Aquarium were sampled periodically from January 2003 through March 2008, at the Aquarium of the Pacific samples were taken in June 2003 and May 2004, and at the New England Aquarium a single sample was taken in March 2005. Vitamin D_3 (25-OH-D) level reported for Myrtle is a single measurement only, not a mean. Turtles in the table are arranged in approximate descending order of mean concentration of 25-OH-D in the blood.

Turtle ID	Age (yr)	Sex	Months indoors	No. of samples	Blood 25-OH-D (nmol/L)				Average weekly vitamin D intake (IU)
					Max.	Min.	Mean	Standard deviation	
Raphael	36	Female	0	2	64	61	62.5	2.1	
Michelangelo	36	Female	0	2	69	61	65	5.7	
Donatello	40	Male	4.5, 17	2	64	46	55	12.7	
Leo	40	Male	11, 24	2	53	45	49	5.7	
509	8–12	Female	8–61	11	36	22	27.3	2.8	1140
171	27–40	Male	30–95	10	37	15	27	7.1	1540
172	27–37	Male	31–95	9	17	5	12.3	2.1	1140
Myrtle		Female	~ 432	1			15		

offered to each turtle daily followed this general pattern: 744 g on Sunday; 2092 g on Monday, Wednesday, and Friday; and 1967 g on Tuesday and Thursday. Food was not offered on Saturday. Quantity of food offered was reduced when fat bulges began to appear around flippers and neck. Thus, during the course of the study, the larger male (171) consumed food equivalent to about 7%–8% of body weight per week (BW/WK), the smaller male (172) consumed about 10%–14% BW/WK, and the female consumed about 13%–20% BW/WK.

An analysis of nutritional composition completed in 2003 showed that the combination of foods contained approximately 0.031 IU of vitamin D per gram. Therefore, each turtle ingested approximately 340 IU of vitamin D per week with their food. Each Sea Tab vitamin contained 400 IU of vitamin D_3. Thus, the largest male's diet was supplemented weekly with 1200 IU of vitamin D, and the 2 smaller turtles were given a weekly supplement of 800 IU of vitamin D. Total vitamin D intake for the larger male was therefore approximately 1540 IU per week (10–12 IU/kg/wk), for the smaller male 1140 IU per week (11–14 IU/kg/wk), and for the juvenile female 1140 IU per week (13–21 IU/kg/wk).

Blood samples for analysis of 25-OH-D were taken from the 3 turtles held at the Shark Reef Aquarium, 4 turtles held at the Aquarium of the Pacific, and 1 at the New England Aquarium. These turtles are identified by number or name in Table 1. Blood samples (1.5 mL) were taken at intervals from January 2003 through March 2008 using a 6-cc syringe with a 20-gauge, 38-mm-long needle. The needle was inserted into the venous sinus at a 30° angle about one-third of the distance from the head to the carapace and one-third of the distance from the dorsal midline to the lateral aspect of the neck. Blood was allowed to clot at room temperature for 30–60 minutes, and serum was refrigerated (~ 40°F) and shipped on ice via overnight courier to the Animal Health Diagnostic Laboratory in Lansing, Michigan, for analysis. In September and October 2007 and September 2008, blood samples for analysis of numerous additional blood parameters were taken from the 3 turtles at the Shark Reef Aquarium and analyzed by Antech Diagnostics, Irvine, California.

Two turtles at the Aquarium of the Pacific exposed to natural sunlight throughout their life were moved indoors where only artificial lighting was available. Their blood was sampled, respectively, at 4.5 and 17 months (Donatello) or 11 and 24 months (Leo) after being moved indoors. Blood samples were also taken from 2 turtles (Raphael and Michelangelo) held in natural sunlight at the Aquarium of the Pacific and from a single female (Myrtle) housed inside under artificial light since being acquired by the New England Aquarium in 1969. Myrtle's blood was sampled 432 months after she was moved indoors. The relationship between blood serum concentration of vitamin D_3 and time spent indoors for all turtles was examined statistically using SPSS Version 7.5.

RESULTS

UVB intensity in the feeding area within a 1.5-inch-diameter circle at the water surface 36 inches directly under new UVB heat lamps was 100 ± 50 μW/cm^2. Light from the bulbs fell on a much broader area of the water surface, but there was no detectable UVB beyond the 1.5-inch-diameter circle directly below the heat lamp. The 50% variation in UVB intensity reflects differences in individual bulbs as delivered from the manufacturer. In spite of the fact that UVB lamps were changed every 6 months in accordance with manufacturer's instructions, intensity degraded rapidly with time. Because the Shark Reef turtles spent little time in the feeding area with any part of their body directly exposed to the 1.5-inch-diameter circle and because the UVB intensity degraded rapidly with time, we concluded that the heat lamps we used were unlikely to have a discernible influence on blood concentrations of 25-OH-D. Nevertheless, use of UVB lamps in the feeding area was continued from January 2003 through May 2008.

Table 2. Comparison of Ca, P, and albumin in the blood of wild green turtles living near the Strait of Hormuz (Hasbun et al. 1998) with turtles held at the Shark Reef Aquarium. Asterisks (*) indicate parameters that fall more than 1 standard deviation from the mean reported for wild turtles. A + or – after a value indicates parameters that fall, respectively, above or below the range reported for wild turtles. Abbreviations are as follows: Ca = calcium, P = phosphorus, AL = albumin. Medium-sized turtles, as defined by Hasbun et al. (1998), have a curved carapace length (nuchal notch to posterior notch) of 70–88 cm, while large turtles have a curved carapace length > 89 cm. Measurements of turtles at Shark Reef were taken with calipers and therefore are straight-line measurements (see Table 3). Turtles of comparable sizes are compared.

Medium-sized turtles

	Near Strait of Hormuz					Shark Reef Aquarium			
						509		172	
	n	Standard deviation	Max.	Min.	Mean	9 Sep 2007	9 Mar 2008	9 Sep 2007	9 Mar 2008
Ca (mg/dL)	10	2.98	14.80	5.12	8.99	7.7	8.2	6.3	6.8
P (mg/dL)	10	1.61	9.90	4.40	7.55	5.4*	6.3	6.2	6.9
AL (g/dL)	7	0.3	2.5	1.6	1.96	2.2	2.1	1.3*–	1.3*–

Large male turtles

	Near Strait of Hormuz					Shark Reef Aquarium (171)		
	n	Standard deviation	Max.	Min.	Mean	9 Sep 2007	7 Oct 2007	9 Mar 2008
Ca (mg/dL)	11	2.60	14.80	5.12	10.46	8.3	8.2	8.1
P (mg/dL)	12	1.92	11.10	4.40	7.11	6.4	5.6	6.7
AL (g/dL)	9	0.3	2.4	1.4	1.97	2.7*+	2.5*+	2.6*+

Vitamin D_3 (25-OH-D) concentrations in the blood of captive green turtles show considerable variability. Table 1 demonstrates that the 2 turtles held outdoors at the Aquarium of the Pacific maintained higher concentrations of 25-OH-D in their blood than any of the turtles held indoors at any of the 3 facilities from which measurements were taken. Furthermore, concentration of 25-OH-D in the blood of the 2 male turtles held indoors at the Aquarium of the Pacific is proportional to the duration of exposure to the indoor environment. In both cases the second sample, taken following longer exposure to the indoor environment, showed lower serum concentration than did the first. Turtles held indoor at the Shark Reef Aquarium consistently had lower levels of 25-OH-D in their blood than did any of the turtles held indoor for shorter periods of time at the Aquarium of the Pacific. Although the Shark Reef female was acquired about 2 years later (May 2002) than were the 2 males, all 3 turtles were fed identical diets and have been exposed to the same light intensity and quality. The smaller male (172) at the Shark Reef Aquarium had the lowest blood concentration of 25-OH-D. Myrtle, a female held indoors at the New England Aquarium since 1969, had a similarly low concentration of 25-OH-D. We have no estimates of probable quantities of vitamin D ingested with food at either the Aquarium of the Pacific or the New England Aquarium.

Blood parameters of green turtles held at the Shark Reef Aquarium do not differ greatly from those seen in wild turtles living near the Strait of Hormuz (Table 2). Most calcium (Ca), phosphorus (P), and albumin (Al) measurements for our turtles fall within 1 standard deviation of those measured for wild turtles, though all also appear to be somewhat below the mean for wild turtles. One phosphorous (P) measurement for a medium-sized turtle at Shark Reef is more than 1 standard deviation lower than the mean for wild turtles. All 3 albumin measurements for the large shark reef male are more than 1 standard deviation higher, and 2 of the 3 albumin measurements for the medium-sized shark reef male are more than 1 standard deviation lower than the mean for wild turtles. None of these parameters appear extreme, and when compared with data taken from 100 wild juvenile turtles from the Bahamas (Bolten and Bjorndal 1992), no exceptional measurements are apparent.

Statistical analysis using SPSS Version 7.5 revealed a negative relationship between time spent indoors and blood serum level of 25-OH-D (Fig. 1). An initial exploratory scatter plot demonstrated what appeared to be a moderate negative linear correlation between 25-OH-D blood concentrations in sea turtles and time spent indoors. An extreme outlier (Myrtle, who had been indoors about 432 months) was identified and eliminated from the data. The relationship between 25-OH-D levels and time spent indoors was then investigated using the Pearson product-moment correlation coefficient. Preliminary analyses consisting of variable histograms and scatter plots of expected vs. observed and standardized residuals of the dependent variable were performed to ensure no violation of the assumptions of normality, linearity, and homoscedasticity (Pallant 2001). This analysis confirmed a moderate negative correlation between months spent indoors and 25-OH-D concentrations ($r = -0.77$, $n = 41$, $p < 0.0001$). The correlation remained moderate to strong when partial correlation was run to explore 25-OH-D levels in association with months

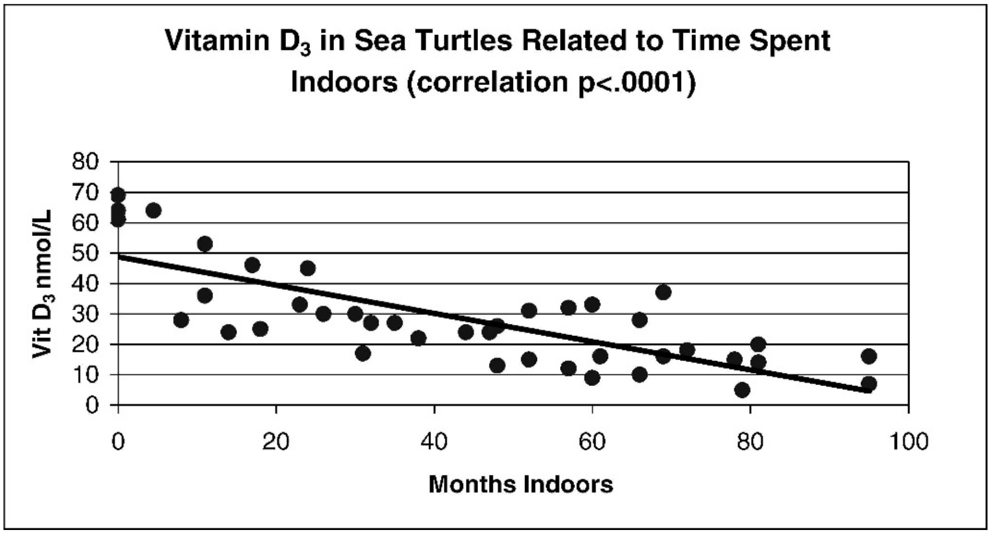

Figure 1. Relationship of blood serum concentration (nmol/L) of 25-OH-D in captive green turtles to time spent indoors without the opportunity to bask in natural sunlight. Data are derived from the 4 green turtles held at the Aquarium of the Pacific (2 samples each) and the 3 green turtles held at Shark Reef (9, 10, and 11 samples; see Table 1). Samples were taken after turtles had spent from 0 to 95 months indoors without an opportunity to bask in natural sunlight.

spent indoors while controlling for individual turtles ($r = -0.82$, $n = 38$, $p < 0.0005$) and gender ($r = -0.76$, $n = 38$, $p < 0.0005$), suggesting that neither specific turtles nor gender have much effect on the strength of the relationship.

A standard multiple regression was used to determine how well time (months spent indoors) predicted 25-OH-D concentrations in the presence of other factors (age, weight, temperature) and if it is the best predictor considering all the other factors. Again, preliminary analyses using collinearity diagnostics indicated that there were no violations of the assumption that multiple correlation with other variables is not high for any of the variables. Results indicated that months spent indoors was indeed the best predictor of 25-OH-D concentrations (−0.77). Comparing the contribution of each independent variable, only months spent indoors is significant ($\beta = -0.90$, $n = 41$, $p < 0.0001$), followed by weight ($\beta = 0.24$, $n = 40$, $p = 0.11$), age ($\beta = +0.17$, $n = 41$, $p = 0.17$), and temperature ($\beta = -0.14$, $n = 34$, $p = 0.25$). Adjusted R^2 was 0.699, suggesting that ~ 70% of the variance in 25-OH-D levels is explained by this model.

DISCUSSION

Green turtles are known to bask both onshore and at the sea surface (Spotila, 2004). Data from other reptiles suggests that the behavior is likely to result in synthesis of 25-OH-D and may provide an essential supplement to vitamin D ingested with food. As with other vertebrates, green turtles are likely to require sufficient quantities of vitamin D to permit normal calcium and phosphorus metabolism, which in turn is essential for normal growth, development, reproduction, maintenance of a healthy skeleton, and normal blood biochemistry. Our limited data suggest that captive green turtles held outdoors and therefore presumably able to bask at will have significantly higher concentrations of 25-OH-D than do turtles held indoors (Table 1). The data also demonstrate (Fig. 1) that when green turtles are housed indoors, away from direct sunlight, blood serum concentrations of 25-OH-D begin to decline after 4–5 months and continue steadily downward for about 6–8 years. The lowest blood serum concentration of 25-OH-D measured was 5 nmol/L, a concentration measured in our small male (172) after 6.5 years of indoor habitation (Table 1). Turtle 172 consistently exhibited low levels of 25-OH-D, yet quantities ingested, based on either total (1140 IU/wk) or relative (11–14 IU/kg/wk) amount, were not markedly different from our other 2 turtles.

Green turtles held at the Shark Reef Aquarium appear to be healthy. For example, in Table 3 we show that body weight and carapace length and width of our mature male green turtles over the past 8 years have remained relatively stable. Our immature female has gained weight, and carapace length and width have increased. Our turtles are also examined at least every 6 months by veterinarians who have detected no disease, abnormal behavior, shell anomalies, or other osteogenic deformities that would suggest clinical consequences of vitamin D insufficiency. Animal curators at the Aquarium of the Pacific and the New England Aquarium also report no symptoms potentially attributable to chronic vitamin D insufficiency. While this study suggests chronic vitamin D insufficiency in green turtles held indoors for years, evidence of the condition may require bone density measurements, attempts to achieve reproduction in captivity, more sophisticated biochemical tests, or more time to allow symptoms to emerge.

Turtle carapaces are subject to a number of growth anomalies, some of which might be caused by inadequate

Table 3. Changes in weight, carapace length, and carapace width of green turtles held at the Shark Reef Aquarium. Initial measurements for the 2 mature males (171, 172) were made on 30 January 2002, and for the immature female (509) on 16 April 2003. Final measurements for all turtles were made on 4 September 2007. Measurements (total of 7–9 measurements distributed throughout the dates indicated) are of straight-line carapace length or width and were made from the anteriormost edge of the carapace to the posterior tip of the longest supracaudal or to the widest point of the marginal scutes.

	Turtle ID								
	171			172			509		
	Weight (kg)	Length (cm)	Width (cm)	Weight (kg)	Length (cm)	Width (cm)	Weight (kg)	Length (cm)	Width (cm)
Initial	133.9	98.3	73.5	104.4	84.5	60.33	59.5	71	61
Minimum	131.7	96.4	73.2	79	84.4	60	54	68	59.3
Maximum	147.1	99.5	74.1	104.4	86	61.8	85.8	76.9	65.7
Final	140.28	99.4	74	86.26	85.4	61	85.8	76.9	65.7

vitamin D (this is sometimes assumed but has not been systematically studied). Kyphotic (humpbacked) and lordotic (groove-backed) turtles are found rarely in a variety of turtle species in nature, but carapace ''pyramiding'' is a common anomaly of captive turtles. It is often attributed to a diet containing excessive protein. Since vitamin D is important in osteogenesis, absence of shell anomalies or other osteogenic deformities might be cited as evidence that the turtles are not ''suffering'' from a vitamin D shortage that has clinical implications. On the other hand, recognizable symptoms attributable to vitamin D insufficiency may become more evident as duration of time spent in captivity increases or as husbandry increasingly focuses on captive reproduction.

The Shark Reef Aquarium has recently built facilities that will allow turtles to bask in direct sunlight for controlled periods. We plan to use these facilities to continue an examination of vitamin D concentrations in blood of captive green turtles. Vitamin D concentrations can probably also be influenced by quantities ingested with food. For our turtles this could readily be tested by increasing numbers of Sea Tab Marine Animal Vitamins fed to each turtle.

It seems clear that green turtles held indoors under relatively low light conditions at the Shark Reef Aquarium, the Aquarium of the Pacific, and the New England Aquarium have significantly lower concentrations of 25-OH-D in their blood than is the case for the 2 turtles held outdoors at the Aquarium of the Pacific. To date, however, there are no data to indicate that adverse consequences result from these seemingly very large differences. Based on what we know of the importance of vitamin D in calcium and phosphorus metabolism for other vertebrates, it would be surprising to find that there are no adverse consequences (Holick, 1989, 2007). Perhaps slow dissolution of the large amount of calcium stored in carapace and bones of green turtles permits captive turtles to remain asymptomatic for long periods of time, especially when they are not subject to the metabolic demands of reproduction. As with the red-eared slider (*T. s. elegans*), it is possible that optimal husbandry of green turtles may require opportunities for basking in sunlight or under appropriate UVB radiation.

This study represents a first step toward understanding desirable levels of 25-OH-D that should be maintained in the blood of captive green turtles. In fact, our data appear to be the first reported for this genus. While considerable information is available about many blood parameters of wild, free-ranging green turtles (Bolten and Bjorndal 1992; Hasbun et al. 1998), we found no specific information about vitamin D concentrations. It will continue to be impossible to place data from captive turtles into their proper context until information about normal levels of 25-OH-D in the blood of wild, free-ranging individuals is collected over a wide geographic area. This study indicates that we need a much better understanding of the physiological consequences and husbandry implications of variations in blood levels of vitamin D in captive turtles. Identifying optimum conditions for husbandry of this threatened species becomes increasingly critical as the marine environment around the world deteriorates.

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