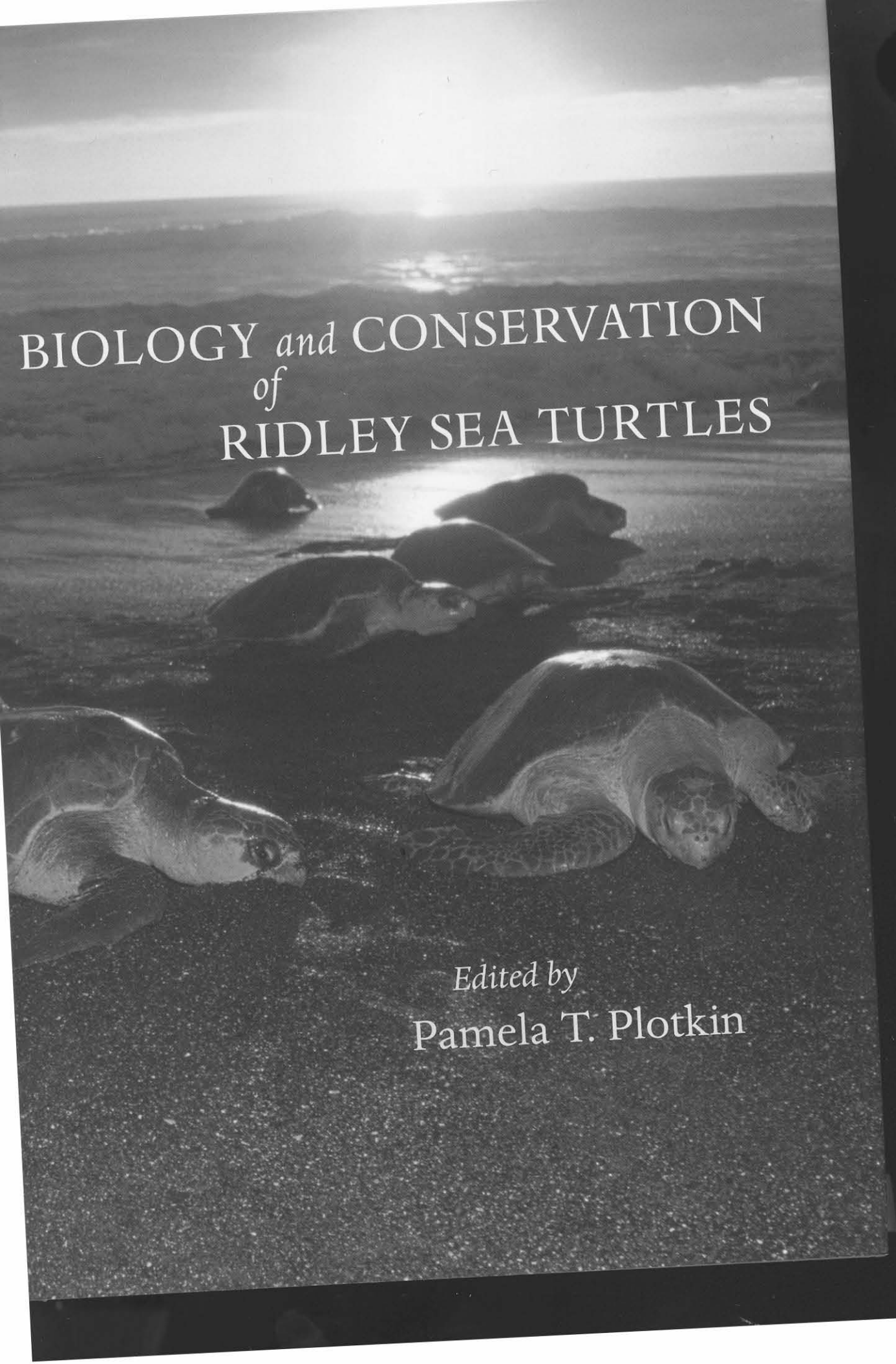
BIOLOGY and CONSERVATION
of
RIDLEY SEA TURTLES
Edited by
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Respiratory and Endocrine Physiology

RIDLEY SEA TURTLES ARE REPRESENTED by two species, the olive ridley (*Lepidochelys olivacea*) and the Kemp's ridley (*L. kempii*). Each species exhibits distinct distribution and abundance, with the olive ridley being significantly more abundant and much more widely distributed. However, these species share the remarkable phenomenon of mass nesting, or *arribada*. An arribada is the synchronized nesting of large numbers of females over a discrete time interval, typically lasting a few days. This mass nesting behavior is limited to the genus *Lepidochelys*. Some olive ridley arribadas may include over 100,000 turtles in a single event (Valverde et al., 1998), in contrast with Kemp's ridley arribadas, which are well under the historical numbers recorded in the 1940s (Márquez-M. et al., 1996). Most of our knowledge of ridley sea turtle biology comes from studies conducted on this terrestrial, ephemeral phase of the turtles' life cycle because of intense interest in this unusual reproductive behavior and the fact that it is easier in the wild to study these migratory animals while on land than during their less accessible, pelagic stages. Despite the technical difficulties of working with large, pelagic turtles in captivity, intense interest in the conservation of ridley turtles has provided unique opportunities for an examination of their metabolic and endocrine physiology. In this chapter, we review our current knowledge of these areas and suggest further directions that might profitably be pursued in nonreproductive ridley physiology.

Respiratory and Acid–Base Balance

Much of the available information on the blood respiratory and acid–base physiology of ridley sea turtles resulted from research with captive-reared Kemp's ridleys. Animals for these studies were raised at the NMFS Galveston Laboratory as part of the Head Start Experiment or were reared in captivity for periods ranging from 1 to 4 years for use as test subjects in turtle excluder device (TED) certification trials. Most of the initial research focused on quantifying the physiological stress associated with involuntary forced submersion. However, the availability of the NMFS Galveston turtles led to additional research on Kemp's ridley sea turtle physiology, on such diverse topics as the anesthetic management of Kemp's ridleys subjected to surgical procedures (Moon and Stabenau, 1996) and identification and characterization of erythrocyte anion exchange in Kemp's ridley (Stabenau et al., 1991b). The following section summarizes the information compiled during the course of these studies.

Submergence Studies

It was suggested in the late 1980s and early 1990s that the commercial shrimp fishery was responsible for significant numbers of sea turtle deaths as a result of incidental capture of turtles during trawling (Henwood and Stuntz, 1987; National Research Council, 1990). In fact, annual mortality estimates ranged from 5,500 to 50,000 Kemp's ridley and loggerhead turtles (*Caretta caretta*) killed in commercial shrimping-related activities. It was proposed that at-sea mortality would be negligible if tow times were reduced to 60 minutes or less (Henwood and Stuntz, 1987; National Research Council, 1990). No information was available in the literature, however, on the physiological consequences of prolonged submergence of sea turtles. Thus, initial studies were designed to quantify the physiological effects of prolonged, forced submergence in Kemp's ridley turtles.

Extended submergence can cause severe acid–base imbalances that could reduce turtle survival. Therefore, lengthy forced submergence experiments were conducted on small numbers of 2- to 4-year-old Kemp's ridleys with congenital flipper or shell deformities. Deformed turtles and numbers used were limited in the experiments because of the possibility that extended submergence might cause severe acid–base imbalances that could lead to death. Although some of the submergence experiments were conducted with noncannulated turtles, for six turtles, the right carotid artery was occlusively cannulated to permit blood sampling without repeated handling. Because a 90-minute tow time would cause significant mortality (National Research Council, 1990), submergence durations of 20, 40, 60, and 80 minutes were planned. All experiments were conducted under appropriate state and federal threatened/endangered species permits to the NMFS.

To perform the first series of experiments, presubmergence blood samples were collected from arterial cannulas, and the turtles were individually submerged in a weighted canvas bag for 20 minutes. Blood samples were then collected immediately postsubmergence and at 50 and 250 minutes postsubmergence. In this experiment and in all of the others below, no more than 4–6% of total blood volume was collected during the serial sampling. Blood pH and blood gases (P_{CO_2} and P_{O_2}) were analyzed with a blood gas analyzer with electrodes thermostatted to turtle body temperature. Intracellular pH was determined by centrifuging an aliquot of blood, removing the supernatant, and freezing the resulting pellet. The sample was then repeatedly thawed and frozen to lyse the erythrocytes and permit measurement of intracellular pH. Blood lactate was determined enzymatically after deproteinating blood with perchloric acid. Plasma Na^+ and K^+ were analyzed by flame photometry, and plasma Cl^- was measured with a chloridometer. Erythrocyte cell water was determined by adding an aliquot of blood to preweighed aluminum foil and drying the sample to a constant weight in an oven at 80°C. The dried blood samples were then reconstituted with nitric acid to permit measurement of the intracellular Cl^- concentrations.

After only 20 minutes, involuntary submergence of 4-year-old 20-kg Kemp's ridley turtles produced a significant and severe respiratory and metabolic acidosis (Table 7.1). Extracellular and intracellular pH and plasma bicarbonate

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significantly decreased, and significant increases in P_{CO_2} (average increase of 24.8 mm Hg) and lactate (average increase of 16.6 mM) were measured (Table 7.1). For comparison, Lutz and Bentley (1985) reported similar lactate increases following a 30-minute submergence of loggerhead turtles. Hochachka et al. (1975) reported lactate increases greater than 35 mM following 2 hours of forced submergence of green sea turtles (*Chelonia mydas*). As shown in Table 7.1, increasing the length of the forced submersion to 40 or 60 minutes in two turtles produced profound and severe respiratory and acid–base imbalances, with extracellular and intracellular pH dropping below 6.5 and P_{CO_2} increasing to almost 150 mm Hg. The consequence of extended submergence was that the turtles were incapable of staying at the surface to ventilate. Thus, they had to be held out of water during the postsubmergence recovery period. Although the turtles eventually recovered from the severe blood acid–base and respiratory imbalance, the blood acid–base data and the turtle postsubmergence behavior suggest that a comparably submerged, incidentally captured turtle at sea that was released "alive" would not survive. Moreover, the physiological consequences of the long-term exposure to the various organs and organ systems were not examined. Subsequent submergence of one cannulated 4-kg Kemp's ridley revealed that turtle size also influenced the magnitude of the acid–base disturbance. Blood pH decreased from 7.36 to 6.5, P_{CO_2} increased from 37.1 to 159.1 mm Hg, and lactate increased from 4.6 to 20.2 mM. The 0.86 drop in blood pH measured in the 4-kg juvenile turtle is over two times the decrease in blood pH measured in 20-kg turtles (average 0.41 units). After 50 minutes, the small Kemp's ridley still had a blood pH of 6.84, suggesting incomplete recovery from the acid–base disturbance. The severity of the acid–base changes measured in this juvenile turtle clearly suggests that a turtle of this size cannot tolerate a 40- or 60-minute submergence without its survival being affected.

It should be noted that the hematological values shown in Table 7.1 are comparable to those found in subsequent studies with non–congenitally deformed Kemp's ridley turtles (E. K. Stabenau, unpublished data). However, definitive conclusions about healthy wild animals cannot be made from these forced submergence studies because these experiments used only a limited number of congenitally deformed turtles. Nevertheless, these results suggest that reduction of shrimp trawl tow times, by itself, would be an ineffective management strategy for a number of reasons. The National Research Council (1990) suggested that restricting tow times to 60 minutes or less in winter and 40 minutes or less in summer may be sufficient to reduce incidental capture mortality. However, the data discussed above reveal that involuntary submergence and turtle size clearly influence the magnitude of the blood acid–base imbalance. Second, the physiological consequences to turtles that may be exposed to multiple periods of extended forced submergence have not been examined. Extended periods of forced submergence of Kemp's ridley turtles would clearly predispose the turtles to dying if the turtles were returned to the water immediately following submergence. Third, enforcing trawl times of any magnitude during any season would be difficult.

Continued at-sea mortality caused by incidental capture of sea turtles in commercial shrimp fishing trawls led the U.S. government to pass regulations in 1987 requiring commercial shrimping vessels to use nets equipped with TEDs (see National Research Council, 1990, for a discussion of TEDs and TED modifications). This also prompted further physiological research projects that were designed to quantify the blood respiratory and acid–base status of Kemp's ridley turtles subjected to involuntary submersion in TED-equipped nets (Stabenau et al., 1991a). TED testing or certification involves exposing turtles to control and candidate TEDs. Through the mid-1990s, 2- and 3-year-old captive-reared Kemp's ridley turtles were used as test subjects in TED trials. The test involved placing a turtle inside a weighted canvas or mesh bag that is transferred from the water's surface to the trawl by means of a messenger line attached to the trawl headrope. Divers then released the turtle into the mouth of the trawl. Each turtle was given 5 minutes to escape the trawl voluntarily; turtles remaining in the trawl after 5 minutes were removed by divers. To quantify the physiological stress associated with TED exposure, presubmergence blood samples were collected from the dorsal cervical sinus of

Table 7.1 Effects of prolonged forced submergence on the blood respiratory, acid–base, and ionic status of 4-year-old, 20.3 ± 2.7–kg Kemp's ridley turtles at 25.8 ± 1.2°C

		Blood-sampling intervals			
Characteristic		Presubmergence	Postsubmergence	50-minute recovery	250-minute recovery
pH_o	20 min	7.50 ± 0.07	7.09 ± 0.20	7.31 ± 0.20	7.57 ± 0.07
	40 min	7.57	6.76	7.21	7.65
	60 min	7.33	6.49	6.90	7.47
pH_i	20 min	7.25 ± 0.09	6.92 ± 0.13	7.05 ± 0.07	7.26 ± 0.03
	40 min	7.21	6.59	6.93	7.29
	60 min	7.10	6.46	6.75	7.21
RBC water (%)	20 min	64.9 ± 0.67	64.7 ± 1.35	65.0 ± 1.41	64.4 ± 0.83
	40 min	64.5	66.1	66.2	64.2
	60 min	66.0	33.0	31.0	35.0
HCO_3^- (mM)	20 min	30.0 ± 6.37	19.5 ± 4.27	17.2 ± 4.59	29.3 ± 5.39
	40 min	30.8	16.6	12.2	34.4
	60 min	40.6	17.6	7.6	22.7
Cl_o^- (mM)	20 min	111.5 ± 3.14	116.6 ± 4.16	114.1 ± 5.61	115.2 ± 4.25
	40 min	115.6	115.3	109.7	109.9
	60 min	116.9	119.0	121.7	121.0
Cl_i^- (mM)	20 min	72.4 ± 1.94	75.4 ± 1.71	74.1 ± 3.60	74.2 ± 3.64
	40 min	74.5	76.2	72.6	70.5
	60 min	87.2	105.9	105.7	84.4
Hematocrit (%)	20 min	29.2 ± 0.87	30.7 ± 3.05	30.4 ± 3.25	29.5 ± 4.04
	40 min	29.1	28.5	30.5	28.0
	60 min	30.5	35.0	35.5	28.5
P_{CO_2} (mm Hg)	20 min	32.8 ± 4.74	57.6 ± 18.78	29.6 ± 6.21	27.0 ± 4.20
	40 min	28.7	105.3	27.3	26.7
	60 min	51.5	149.4	37.3	32.7
Lactate (mM)	20 min	1.35 ± 0.87	17.9 ± 5.65	18.4 ± 6.30	5.2 ± 4.05
	40 min	0.9	29.7	27.3	6.9
	60 min	4.1	20.8	20.7	16.8
Na_o^+ (mM)	20 min	139.8 ± 2.45	148.2 ± 3.22	142.9 ± 5.29	141.5 ± 5.70
	40 min	146.4	155.6	147.2	142.7
	60 min	139.9	161.8	146.9	144.3
K_o^+ (mM)	20 min	3.5 ± 0.62	5.9 ± 1.04	4.2 ± 1.12	3.8 ± 0.45
	40 min	3.4	6.0	4.0	3.7
	60 min	5.7	14.2	9.3	8.1
P_{O_2} (mm Hg)	20 min	82.6 ± 11.78	57.2 ± 27.70	75.1 ± 22.20	74.4 ± 24.48
	40 min	85.0	35.2	97.7	67.4
	60 min	42.9	6.5	38.4	30.1

Note: Turtles were confined in weighted canvas bags and submerged for 20 ($n = 4$), 40 ($n = 1$), or 60 ($n = 1$) minutes. The subscripts i and o represent intracellular and extracellular, respectively. Where appropriate, data are expressed as mean ± SD.

Kemp's ridley turtles as described by Owens and Ruiz (1980). The shortest submersion duration in the original study was under 2.7 minutes, and the maximum time any turtle spent underwater was 7.3 minutes. This included the time to get the turtle to the headrope, the maximum exposure time to escape the TED, and the time to reach the surface after exiting the TED. Turtles were immediately returned to the trawling vessel for collection of postsubmergence blood samples. Blood pH and P_{CO_2} were analyzed with a commercial blood gas analyzer with elec-

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trodes thermostatted at 37°C. The data were adjusted to turtle body temperature with requisite correction factors for sea turtle blood (Stabenau and Heming, 1993). Blood and plasma lactate and Na^+, K^+, and Cl^- were analyzed as described above.

As a result of these experiments, it soon became apparent that even TEDs did not fully alleviate the physiological impacts of trawling on sea turtles. Short-term involuntary submergence of Kemp's ridley turtles in TED-equipped nets produced significant blood respiratory and metabolic derangements. Specifically, submergence stress produced significant decreases in blood pH and significant increases in blood lactate, P_{CO_2}, and plasma K^+. Although recovery from the acid–base disturbance was not measured in these studies, subsequent experiments in 1993 and 1994 revealed that a period of at least 3 hours was required for recovery of blood variables to prestress levels. The physiological data collected during the TED certification trials indicated that short-term (i.e., 7.5 minutes or less) forced submergence of Kemp's ridley turtles exceeded the animals' aerobic capacity. It is unclear, however, if there were long-term consequences to forced submergence. For example, no information is available on whether turtles become more susceptible to repeated submergence in TED-equipped nets or whether turtles resume normal diving and feeding behaviors following forced submergence (for a description of wild Kemp's ridley diving submergence duration and swimming speed, see Renaud [1995]).

In 1994, additional TED certification trials were conducted with yearling Kemp's ridley and loggerhead turtles. The objective was to assess whether loggerhead turtles could be used as surrogates for Kemp's ridley turtles in annual TED tests and certification trials and to determine the physiological stress of smaller turtles during TED-test forced submersion. Forced submergence experiments (submergence duration = 7.5 minutes) were conducted as described above, with the exception that the turtles were held in large in-water pens 30 days before the TED trials to simulate semiwild conditioning. In addition, blood samples were collected 3 and 6 hours after submergence. Yearling Kemp's ridley and loggerhead turtles exhibited nearly identical pre- to postsubmergence changes in blood pH and lactate. Presubmergence pH in Kemp's ridley and loggerhead turtles was 7.53 ± 0.06 (mean $\pm$ SD) and 7.57 ± 0.05, respectively. The postsubmergence blood pH measured immediately after the turtle surfaced decreased to 7.06 ± 0.03 in ridleys and 7.16 ± 0.11 in loggerheads. Three hours after submergence, the blood pH was 7.55 ± 0.04 and 7.52 ± 0.04 in ridley and loggerhead turtles, respectively. No differences in blood pH were detected 6 hours postsubmergence when compared to that measured in presubmergence samples, suggesting that turtles exhibited a full recovery from the forced submergence. These data, in combination with comparable blood lactate loads following submergence, indicated that yearling loggerhead turtles could serve as surrogates for yearling Kemp's ridley turtles during TED testing and certification. However, extending this species comparison beyond yearling turtles has not been investigated. More importantly, no study has examined the physiological effects of forced submergence in TED-equipped nets as a function of turtle size.

Recently, Stabenau and Vietti (2003) examined the physiological effects of multiple submergences on the blood respiratory, acid–base, and ionic status of 6- to 7-kg loggerhead turtles. The purpose of these experiments was to determine whether repeated forced submergence induces progressive, significant blood acid–base disturbances. Experiments were conducted initially under laboratory conditions by confining turtles in weighted canvas bags for three 7.5-minute submergences with a 10-, 42-, or 180-minute "rest" interval between successive submergences. Field experiments were also conducted by exposing turtles under TED-test conditions, with the exception that divers held the exit door closed for 5 minutes. Thus, the total time underwater for each turtle was 7.5 minutes. Blood samples were collected before and immediately after each submergence and 3 hours after the last submergence. No turtles died during the course of these studies. The data revealed that (1) the initial submergence produced a severe metabolic and respiratory acidosis in all turtles, (2) successive submergences produced significant changes in blood variables (e.g., lactate, pH, P_{CO_2}), although the magnitudes of the imbalances were reduced as the number of submergences increased, (3) significant water

movement into and out of red blood cells occurred during and after multiple forced submergence, (4) increasing the interval between successive submergences permitted greater recovery of blood homeostasis, (5) similar changes were not observed in nonsubmerged control turtles that had the same serial blood sampling regimen, and (6) repetitive submergence in TED-equipped nets (assuming proper installation and use) would not cause death provided that the animals had an adequate rest interval at the surface between submergences (Stabenau and Vietti, 2003). Comparable information is not available for any other size of turtle or any other sea turtle species and warrants investigation.

Additional Physiological Studies

Much of the information described above was concerned with the physiological effects of forced submersion, but additional experiments have been conducted with blood and other tissues from Kemp's ridley turtles. The following section summarizes the results of these studies.

Red Cell Ion Transport

The presence of erythrocyte anion-exchange protein (i.e., Band 3) permits membrane HCO_3^- transport in a number of species primarily through Na-independent HCO_3^-/Cl^- exchange. Erythrocyte HCO_3^- in many species plays a critical role in CO_2 transport and regulation of plasma and intracellular pH. Although the mechanism of HCO_3^-/Cl^- exchange has been described in a wide variety of vertebrates, less information is available on HCO_3^-/Cl^- exchange in reptilian red blood cells. In turtles, the presence of Band 3 protein for erythrocyte HCO_3^-/Cl^- exchange has been demonstrated in the slider turtle *Pseudemys scripta* (Drenckhahn et al., 1987) and the Kemp's ridley (Stabenau et al., 1991b). In the latter study, it was determined that Kemp's ridley turtle erythrocytes contain 4×10^6 copies of Band 3 protein per cell, or 8,000 copies of Band 3 per square micrometer (Stabenau et al., 1991b). For comparison, human and trout erythrocytes possess approximately 7,000 and 30,000 copies of Band 3 per square micrometer, respectively (Knauf, 1979; Romano and Passow, 1984). Kinetic analysis of the HCO_3^-/Cl^- transporter revealed that erythrocyte anion exchange in Kemp's ridley turtles may be a potentially rate-limiting step for capillary CO_2 exchange (Stabenau et al., 1991b). This limitation may be directly related to the results of the previous studies that showed that short- or long-term forced submersion of Kemp's ridley turtles leads to substantial increases in blood P_{CO_2} from normal values of 35–40 mm Hg to values well above 100 mm Hg (Table 7.1). If capillary HCO_3^-/Cl^- exchange is rate-limiting in the elimination of elevated blood CO_2 following submersion stress, then postcapillary erythrocyte anion exchange would continue and produce significant changes to arterial blood P_{CO_2} and pH (Stabenau et al., 1991b). More recently, Stabenau and Vietti (2003) proposed that loggerhead turtle erythrocyte HCO_3^-/Cl^- and Na^+/H^+ exchangers function as regulatory volume-increase mechanisms. Na^+ and Cl^- enter osmotically shrunken erythrocytes through the respective transporters, while H^+ and HCO_3^- leave the cells as the countertransported ions. Extracellular H^+ and HCO_3^- then combine to form CO_2 and H_2O. The net result is osmotically obliged water entry into the shrunken erythrocytes, which causes the cells to swell. It is possible that similar ion transport mechanisms are present in Kemp's ridley turtle erythrocytes.

HENDERSON–HASSELBALCH CONSTANTS. A common theme in many of the studies mentioned thus far is the measurement of blood P_{CO_2} or plasma HCO_3^-. However, it is not always possible to measure plasma HCO_3^- concentration or to utilize blood gas analyzers with pH, P_{CO_2}, and P_{O_2} electrodes thermostatted to turtle body temperature. Thus, temperature correction factors of known physiological constants are required for dealing with cold-blooded animals. Mammalian constants are commonly used in analyses of the acid–base status of nonmammalian species despite evidence that these practices produce misleading results (Stabenau and Heming, 1993). Stabenau and Heming (1993) determined the constants of the Henderson–Hasselbalch equation, α_{CO_2} and pK_a, over a 20–30°C temperature range for Kemp's ridley turtle blood and plasma. These constants are typically used in the Henderson–Hasselbalch equation, $pH = pK_a + \log (HCO_3^-/\alpha_{CO_2} \cdot P_{CO_2})$ to calcu-

late blood pH, P_{CO_2}, values based on temp found that use of cla values of α_{CO_2} and p Kemp's ridley turtle malian-derived consta ses of the effects of t turtle blood and plasm

BLOOD AND MUSCL ERTIES. Although s available in the literatu age properties of green back (*Dermochelys coria* Lutz, 1982; Lutcavage about these variables Stabenau and Heming the blood O_2 dissociat ridley turtles exhibited with a P_{50} of 31.2 ± 0.3 The Kemp's ridley turtl reported for green turt 1982) but was considera sured for loggerhead Lutz, 1982). Neverthele bin concentration is sim ley, loggerhead, and gree Heming, 1994; Lapenna significantly less than tha (Lutcavage et al., 1990). concentration is $3.1 \pm 0.$ the Kemp's ridley turtle (1994) and 2.9 and 4.9 mg gerhead (Lutz and Bent back (Lutcavage et al., 19 These studies revealed th ridley turtle muscle, bloo 4.7%, 24.8–35.0%, and 60 For comparison, Lutz a ported that O_2 stores in were 3.6% in the muscle, 71.6% in the lung. Taken indicate that the diving water coastal sea turtle Kemp's ridley and loggerh by low blood and tissue C to deep-diving leatherba these animals have a reliar (Stabenau and Heming, 19 found that Kemp's ridley bicarbonate buffer capacity

late blood pH, P_{CO_2}, or HCO_3^- or to back-correct values based on temperature differences. It was found that use of classical mammalian-derived values of α_{CO_2} and pK_a was not appropriate for Kemp's ridley turtle plasma. Specifically, mammalian-derived constants would confound analyses of the effects of temperature or pH on sea turtle blood and plasma.

BLOOD AND MUSCLE O_2-CARRYING PROPERTIES. Although substantial information is available in the literature on the blood O_2 storage properties of green, loggerhead, and leatherback (*Dermochelys coriacea*) turtles (Lapennas and Lutz, 1982; Lutcavage et al., 1990), less is known about these variables in Kemp's ridley turtles. Stabenau and Heming (1994) determined that the blood O_2 dissociation curves from Kemp's ridley turtles exhibited a classic sigmoidal shape with a P_{50} of 31.2 ± 0.3 at a hematocrit of 29%. The Kemp's ridley turtle P_{50} was similar to that reported for green turtles (Lapennas and Lutz, 1982) but was considerably less than that measured for loggerhead turtles (Lapennas and Lutz, 1982). Nevertheless, the blood hemoglobin concentration is similar among Kemp's ridley, loggerhead, and green turtles (Stabenau and Heming, 1994; Lapennas and Lutz, 1982) and is significantly less than that of leatherback turtles (Lutcavage et al., 1990). The muscle myoglobin concentration is 3.1 ± 0.84 mg $\cdot$ g^{-1} of tissue in the Kemp's ridley turtle (Stabenau and Heming, 1994) and 2.9 and 4.9 mg $\cdot$ g^{-1} of tissue in the loggerhead (Lutz and Bentley, 1985) and leatherback (Lutcavage et al., 1990) turtles, respectively. These studies revealed that O_2 stores in Kemp's ridley turtle muscle, blood, and lung were 3.6–4.7%, 24.8–35.0%, and 60.3–75.2%, respectively. For comparison, Lutz and Bentley (1985) reported that O_2 stores in the loggerhead turtle were 3.6% in the muscle, 24.8% in the blood, and 71.6% in the lung. Taken together, these results indicate that the diving capacity of shallow-water coastal sea turtle species, such as the Kemp's ridley and loggerhead turtles, is limited by low blood and tissue O_2 stores as compared to deep-diving leatherback turtles and that these animals have a reliance on lung O_2 stores (Stabenau and Heming, 1994). The authors also found that Kemp's ridley turtles possess a non-bicarbonate buffer capacity of 19.7 slykes. Many reptile species have significantly higher blood-buffering capacities (Butler and Jones, 1983). The relatively low buffer capacity in Kemp's ridleys may reveal why this species exhibits such substantial and significant changes in blood pH following forced submergence, even for relatively short periods. It is unknown, however, if significant changes in blood pH are observed following voluntary diving by Kemp's ridleys.

ANESTHESIA. Although Butler et al. (1984) and Shaw et al. (1992) utilized the inhalational anesthetics halothane and isoflurane in green and loggerhead turtles, respectively, limited information was available on the anesthetic management of Kemp's ridley turtles. Thus, Moon and Stabenau (1996) examined the anesthetic and postanesthetic management of Kemp's ridley turtles undergoing a fairly invasive surgical procedure. Anesthetic induction with isoflurane (3.4 ± 0.3% in 2 L of carrier gas/min) followed orotracheal intubation. Induction occurred rapidly in 7 ± 1 minutes. The carrier gases during induction were either 100% O_2, 5% CO_2:95% O_2, or 21–40% O_2 (79% or 60% N_2, respectively). No differences in induction time were detected with the various carrier gases. The right carotid artery was occlusively cannulated with polyethylene tubing during anesthesia, and a second surgery was performed 7–10 days later to remove the cannula. Pulse rate was monitored throughout the study with an ultrasonic Doppler flow probe (Parks Medical Electronics, Cherry Hill, NJ) placed over the femoral triangle, thoracic inlet, caudodorsal aspect of the front flippers, or dorsal cervical sinus (Moon and Stabenau, 1996). The preoperative pulse rate was 34 ± 3 beats/min, 15 ± 1 beats/min intraoperatively and during initial recovery, and 50 ± 2 beats/min when awake. Sudden tachycardia was an excellent predictor of the "awake" state in Kemp's ridley turtles. Blood pressures (systolic/diastolic in mm Hg) were $31 \pm 6/20 \pm 4$, $40 \pm 4/25 \pm 3$, and $46 \pm 4/39 \pm 2$, respectively, during the intraoperative, recovery, and awake phases. The turtles became significantly acidotic as a result of an elevated blood lactate concentration during the early and late recovery phases. The duration of the recovery phase (e.g., time of unresponsiveness before being classified as awake) was 241 ± 31 minutes. This study revealed that inhalational anesthetics

could be used for safe and rapid anesthetic induction of Kemp's ridley turtles, and the authors also provided previously unavailable intraoperative vital signs.

ENTANGLEMENT NET CAPTURE STRESS. It must be mentioned that all of the physiological respiratory and acid–base studies described in this review thus far utilized captive-reared Kemp's ridley turtles. More recently, Hoopes et al. (2000) examined the physiological effects of capturing wild Kemp's ridley turtles in entanglement nets. In this study, turtles captured in entanglement nets were placed into in-water cages or on-shore holding tanks. Recovery from capture was then monitored via blood samples collected from turtles immediately postcapture, and at 1, 3, 6, 10, 24, and 48 hours postcapture, depending on turtle size. Larger turtles had more blood samples collected as a result of increased availability. Blood lactate and catecholamine concentrations were measured from each turtle in addition to plasma Na^+, K^+, and Cl^- concentrations. The data revealed that capture in entanglement nets produced a substantial blood metabolic disturbance as indicated by elevated plasma lactate concentrations. Increased plasma norepinephrine, epinephrine, and K^+ concentrations were also detected in capture samples. Although it may have been anticipated that entanglement netting would produce significant changes in blood parameters, an unanticipated result was that the recovery protocol had a significant influence on blood parameters. Specifically, placement of captured turtles into on-shore holding tanks resulted in additional significant increases in the plasma lactate concentrations, whereas turtles in the in-water cages exhibited no changes to the plasma lactate after capture. In addition, the lactate concentrations declined to less than 1 mM by 6 and 10 hours postcapture for turtles in the in-water cages and holding tank treatments, respectively. The decline in lactate over time suggests that repetitive serial blood sampling of wild Kemp's ridleys did not adversely affect results. Thus, Hoopes et al. (2000) clearly showed that sea turtle biologists must be cognizant of physiological disturbances caused by capture protocols and that recovery of blood homeostasis is influenced by postcapture holding protocols.

Summary

The availability of captive-reared Kemp's ridley sea turtles has provided respiratory and reproductive physiologists with ample opportunities to perform many valuable experiments that otherwise would not have been possible. In fact, other than the study by Hoopes et al. (2000), there are no studies on the respiratory and acid–base status of blood and other tissues from wild Kemp's ridley or olive ridley turtles. The data from the present review indicate that ridleys have a remarkable ability to tolerate severe acid–base disturbances. In the forced submergence studies, a drop in blood pH of almost 1 full unit and an increase in blood P_{CO_2} to values over 150 mm Hg were measured. However, the long-term physiological consequences of extended forced submergence are unknown. Surviving an extended forced submergence is only part of the battle. The turtle must also have the capacity to resume normal activities such as feeding, diving, and reproduction, in a timely fashion. Importantly, turtles must not be subjected to resubmergence. Finally, comparable physiological information is unavailable for wild Kemp's ridley and olive ridley turtles and warrants investigation.

Endocrinology

Endocrinology, the study of the synthesis, secretion, and physiological actions of hormones, has historically focused on model species that are readily available for experimental study. Hormones are stored in endocrine glands and circulate in the blood in minuscule amounts. Therefore, large numbers of animals generally are needed to supply adequate tissue for extraction and purification of hormones as well as for biochemical studies of hormone actions at target cells. Because blood hormone concentrations change dynamically over minutes to hours, frequent blood sampling from captive animals is necessary to most effectively describe hormone response to experimental manipulations. Sea turtles, comprising rare and endangered species living in remote locations with limited availability in captivity, would thus seem to represent a poor choice for studies of endocrine physiology.

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It is therefore surprising that sea turtles are among the best-studied reptiles in terms of their endocrinology. This has been motivated in part by the importance to conservation of the understanding of the regulation of reproduction, metabolism, and stress responses (Owens, 1997). Additionally, the large body size of sea turtles facilitates collection of blood samples (Owens and Ruiz, 1980), which are readily obtained from nesting females and captive-reared animals. Captive rearing programs have served as a source of blood samples for hormone measurement and pituitary tissue for hormone purification. Animals from the Cayman Turtle Farm, for example, have provided tissue as a source for purification of pituitary hormones (Licht and Papkoff, 1985; Yasuda et al., 1989, 1990), leading to major advances in our understanding of the regulation of growth and reproduction in both marine and freshwater turtles. They have also served as subjects for studies of behavioral endocrinology (Rostal et al., 1998). Development and application of endocrine techniques have thus progressed more rapidly for sea turtles of the family Cheloniidae than for other reptilian families. However, their status as threatened or endangered species and the remote location of many natural populations have limited application of the invasive techniques of endocrinology primarily to measurement of circulating hormones, particularly in ridley turtles. Because animals are generally unavailable for surgical ablation, hormone administration, or tissue collection for in vitro studies, no information is available on hormone biosynthesis, metabolism, or receptor physiology.

Techniques, primarily radioimmunoassay (RIA), have been developed for the purification or measurement of six sea turtle pituitary hormones: the neurohypophyseal peptide hormone arginine vasotocin (AVT) and its associated carrier protein neurophysin (NP; Licht et al., 1984; Figler et al., 1989), the three glycoprotein hormones, follicle-stimulating hormone (FSH), luteinizing hormone (LH), and thyrotropin (TSH, MacKenzie et al., 1981; Licht and Papkoff, 1985), and the two peptide hormones, growth hormone (GH; Yasuda et al., 1989) and prolactin (PRL; Chang and Papkoff, 1985; Yasuda et al., 1990). Of these six, only the RIA for AVT was developed specifically for ridley turtles. The other RIAs (developed for *Chelonia* of *Chelydra* hormones) can detect hormones in blood or pituitary tissue of several turtle genera (both freshwater and marine species), presumably because of the structural similarity of the pituitary hormones among turtles (Licht, 1978). The RIAs for FSH and LH have been used to study endocrine regulation of sea turtle reproduction (Rostal, Chapter 8). The dynamic changes in circulating levels of these hormones associated with ovulation appear conserved in all sea turtle species examined, including ridleys (Owens, 1997; Rostal, Chapter 8). Although an RIA for turtle GH has been successfully employed in the study of the regulation of GH secretion from pituitaries of freshwater turtles (Denver and Licht, 1989, 1990), comparable studies have not been performed in sea turtles. Likewise, the PRL assay can be used to detect in vitro PRL secretion from adult (but not juvenile) freshwater turtle pituitaries (Preece and Licht, 1987; Denver and Licht, 1988) but has not been applied for sea turtle blood or tissues. These studies have demonstrated the practicality of development of techniques for pituitary hormone measurement in sea turtles. With the advent of modern molecular approaches, it should be possible to develop methods for the measurement of pituitary hormones using minute amounts of tissue from any sea turtle species.

Fundamental studies of pituitary hormone function may benefit from an examination of unique aspects of sea turtle physiology and behavior. As an example, the mass nesting behavior of ridley turtles (arribada) has provided an ideal opportunity to examine the endocrine regulation of egg laying in reptiles. Because a large number of olive ridleys nested within 24 hours on a single beach, Figler et al. (1989) were able to obtain replicated blood samples from nine defined stages of oviposition. These samples were used to demonstrate that AVT undergoes a transient elevation in the blood coincident with its proposed physiological action, oviductal contraction (Figler et al., 1989). This study produced such a clear picture that it is included in a leading textbook of endocrinology (Hadley, 1999) as an illustration of the function of AVT in oviposition. The longevity of sea turtles makes them particularly intriguing for the examination of the role of GH in the regulation of growth in

reptiles, whereas unique aspects of their reproductive biology (migration, high fecundity, nest site fidelity, mass nesting behavior) make them of interest in the study of the evolution of the role of PRL in reproduction. PRL has been implicated in the regulation of the behavior associated with nesting in precocial bird species (Goldsmith, 1983), suggesting it as an intriguing candidate in the regulation of mass nesting behavior in ridleys. PRL has also been implicated in the regulation of ion transport and water permeability of membranes. The endocrine regulation of salt and water balance, including the regulation of calcium metabolism, is unknown in sea turtles (Lutz, 1997). Studies of the osmoregulatory endocrinology of sea turtles, when compared to marine birds that share similar salt excretory organs, would be of interest in identifying common endocrine adaptations to the marine environment.

Techniques for measurement of protein hormones have been limited in their application to sea turtles because of their requirement for collections of fresh tissues and laborious biochemical purification, but other hormones that are conserved in structure across vertebrate classes are more easily measured using commercially available reagents. Specifically, techniques developed for the measurement of steroid hormones, produced by reproductive organs and the adrenal gland, and thyroid hormones, from the thyroid gland, have been employed for sea turtle blood following relatively simple validation. Reproductive steroid hormones in ridley turtles are discussed in detail in Chapter 8. Here, we focus on two endocrine glands, the adrenal and thyroid, that represent the only nonreproductive peripheral endocrine glands examined to date in ridley turtles. Studies in diverse vertebrate species, including freshwater turtles, indicate that these two endocrine glands are controlled by similar endocrine pathways, initiating with neurohormones produced in the hypothalamus, that regulate production and secretion of pituitary hormones (Fig. 7.1).

Adrenal Physiology

STRESS SYSTEM AND STRESS RESPONSE. Survival of a species is dependent on the ability of the individuals to monitor changes in the environment and respond adaptively. In vertebrates, monitoring of environmental changes is under strict control of a well-developed and complex central nervous system (CNS). The CNS is ca-

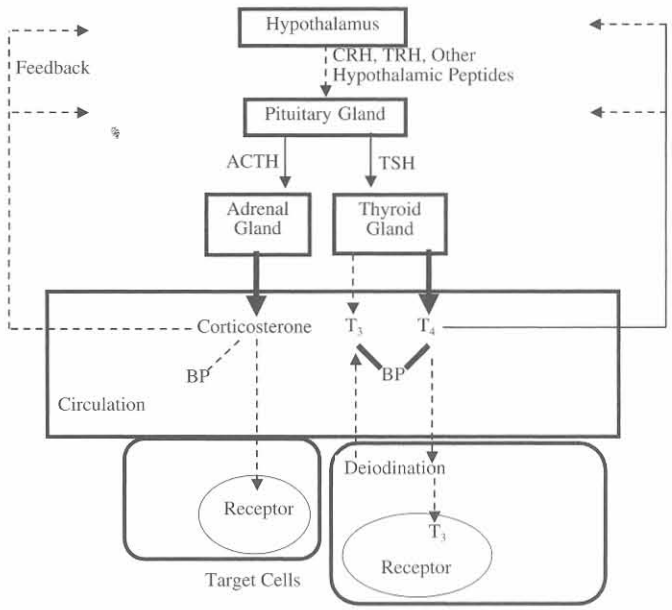


Fig. 7.1. General model for the hypothalamic-pituitary-adrenal and hypothalamic-pituitary-thyroid axes, depicted to illustrate similarities. Heavy lines indicate components known to exist in ridley turtles, thin solid lines indicate components known to exist in other sea turtle species, and dashed lines indicate components not yet proven to exist in sea turtles. ACTH, adrenocorticotropic hormone; BP, blood binding proteins; CRH, corticotropin-releasing hormone; T_3, 3,3′,5-triiodothyronine; T_4, thyroxine; TRH, thyrotropin-releasing hormone; TSH, thyrotropin.

pable of sensing changes in the external as well as in the internal environment, integrating this information, and producing an appropriate response. Frequently, environmental changes disrupt the homeostasis of the organism, requiring modifications in cellular activity to ensure appropriate continued function. Thus, CNS output, manifested through the activation of peripheral effectors such as the adrenal medulla and cortex, has as its primary objective the regulation of homeostatic mechanisms that allow the organism to respond adaptively to environmental changes. The disruption of homeostasis is termed "stress," and the specific stimuli that elicit such disruption are termed "stressors."

Before we engage in a discussion of ridley stress endocrinology, it is important to generate an appropriate perspective by describing the broader stress response of vertebrates, much of which is known from mammalian studies. In the past two decades the field of stress endocrinology has seen substantial conceptual advances that allow us to better understand the physiological and adaptive significance of the stress response. One such advance is the concept of the "stress system." The stress system comprises specific brain areas and peripheral effectors that elicit adaptive behavioral, neuroendocrine, and physiological responses aimed at promoting adaptation and survival (Chrousos and Gold, 1992). When a stressor is perceived, a specific cascade of neural networks is activated. Neural activation in response to a stressor has been demonstrated by the increased transcription of immediate early genes, such as the proto-oncogenes *c-fos* and *c-jun,* among others (Senba and Ueyama, 1997). Activated brain areas include diencephalic and brainstem nuclei, of which the hypothalamic paraventricular nuclei play a central role in regulating the activity of the hypothalamo-pituitary-adrenal (HPA) axis (Fig. 7.1). The HPA axis, along with the brain–adrenal medulla (catecholamine) branch, is one of the most important peripheral components of the stress system.

In classical stress endocrinology, the HPA axis is activated by a broad array of internal and external environmental variables. The unifying factor that stimulates the activation of the axis is the disruption of systemic homeostasis (Chrousos and Gold, 1992). Homeostatic disruptors constitute a stressor and induce glucocorticoid release. Glucocorticoids exert multiple effects in the organism given the ubiquitous presence of their receptors. A simplified list of these effects includes antiinflammatory, antiimmune, antireproductive, and hyperglycemic functions (Widmaier, 1990; Sapolsky et al., 2000). On the basis of these actions, it has been suggested that glucocorticoids suppress the general stress response to prevent the organism from overreacting in a pathological fashion to the stressor (Munck et al., 1984; Sapolsky et al., 2000). Aside from how glucocorticoids achieve their effects, it is widely accepted that one of their main functions is the promotion of survival (Darlington et al., 1990; Sapolsky et al., 2000; Wingfield and Kitaysky, 2002). Thus, increases in blood concentration of glucocorticoids represent a signal that the organism has triggered its adaptive mechanisms in response to a stressor.

As one of the first neuroendocrine signals during the stress response, hypothalamic corticotropin-releasing hormone (CRH) is secreted into the hypothalamo-pituitary portal circulation (Fig. 7.1). Here CRH stimulates pituitary corticotropes to synthesize and release adrenocorticotropic hormone (ACTH) into the systemic circulation. ACTH then stimulates the release of glucocorticoids from the adrenal cortex (Fig. 7.1). In most mammals and fish, cortisol is the main glucocorticoid, whereas in rodents, birds, amphibians, and reptiles, corticosterone appears to be the main glucocorticoid (reviewed by Sandor et al., 1976). First attempts to identify glucocorticoids in ridley turtles were inconclusive (Chester Jones et al., 1959), and more recent attempts to measure cortisol in sea turtle blood have been unsuccessful (A. Aguirre, personal communication). However, corticosterone is well established as the primary corticosteroid in reptiles (Sandor et al., 1976) and has now been measured in response to a variety of putative stressful stimuli in ridley turtles.

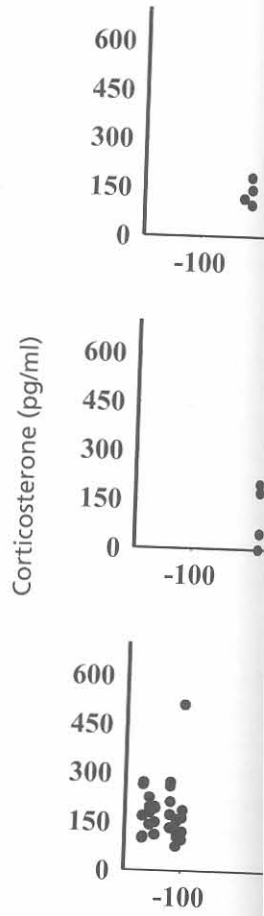
STRESS AND RIDLEY TURTLES. Because the genus *Lepidochelys* includes the most, as well as the least, abundant populations of sea turtles, it is striking that so little is known of their stress endocrinology. It is important that we under-

stand how these turtles adapt to stress not only to advance our knowledge of their biology but also to develop more complete and effective approaches to protecting these reptiles. One reasonable approach would be to conduct stress studies on the more abundant olive ridley for comparison or extrapolation to the more endangered Kemp's ridley. Fortunately, the availability of juvenile Kemp's ridleys at the NMFS Galveston Laboratory used in the head-start experiment has provided rare opportunities for studies of adrenal physiology in this species as well. The following pages review our current knowledge of the ridley endocrine stress physiology based on published and unpublished reports and data. Reviewed accounts on ridley stress physiology, combining field and laboratory-based approaches as well as captive-raised and wild sea turtles, have produced an interesting but as of yet incomplete picture of this field.

An important first step in piecing together the endocrine stress response in ridleys was the characterization of basal circulating glucocorticoid concentrations in the wild. Because corticosterone plays an important role in the control of metabolic functions and undergoes daily fluctuations in response to endogenous rhythms (Mizock, 1995), adrenal output may change significantly in a dynamic fashion. To prevent misinterpretation of experimental outcomes, it is also essential to determine basal fluctuations in blood corticosteroid concentration in each particular species. To address this concern, daily basal corticosteroid concentration was measured in adult, free-ranging olive ridleys off Nancite, an arribada nesting beach in Costa Rica (Valverde et al., 1999). In this initial study, 10 females were captured in the water at each of four times during the day: 0600, 1200, 1800, and 2400. Mean corticosterone concentration remained below 0.2 ng/ml throughout this 24-hour period, with no indication of daily variability. These results were further supported by the sampling of over 100 female olive ridleys randomly captured off Nancite Beach at different times during the peak nesting season. Nearly all turtles exhibited blood corticosterone concentration under 0.6 ng/ml (Fig. 7.2). These data are in agreement with basal blood corticosterone concentration measured in green turtles during the nesting process (Jessop et al., 1999). Interestingly, these basal values for ridley and green sea turtles are well below those described for the gopher tortoise, *Gopherus polyphemus* (Ott et al., 2000), and suggest a hypoactivity of the ridley and green HPA axes. It is important to note that only few published studies provide evidence for, or of lack of, a daily cycle in basal circulating corticosteroid in sea turtles.

The first study of adrenal corticosteroids in the context of the stress response in wild olive ridleys is that by Schwantes (1986). In this study conducted at La Escobilla Beach, in Oaxaca, Mexico, three to eight females were sampled at each of nine different stages of nesting: stranding, beginning nest, body pit, egg chamber, first egg, midclutch, last egg, covering, and returning. Circulating corticosterone concentration remained below assay detectability (<1 ng/ml) throughout the nesting process. These results are similar to basal concentration of corticosterone in the green during the same nesting phases (Jessop et al., 1999). In addition, Schwantes (1986) provided the first demonstration of the functionality of the ridley HPA axis by sampling wild adult olive ridleys held at the former slaughterhouse in San Agustinillo in Oaxaca. Males and females held at the slaughterhouse exhibited mean corticosterone concentrations ranging from 4 to 10 ng/ml, 3- to 50-fold greater than those concentrations observed in animals nesting, mating, or basking offshore. Circulating corticosterone was significantly higher in mating males than in mating females, and in stressed males relative to stressed females held at the slaughterhouse. However, sexual differences were not apparent in basking animals. It is important to point out that it was not possible to determine whether males and females had been exposed to the same treatment at the slaughterhouse. This makes it difficult to ascertain whether differences in corticosterone concentration found in these turtles represent a form of sexual dimorphism. Nevertheless, these results suggest that stress, but not nesting, is an effective activator of the ridley HPA axis.

A contrasting study (Valverde et al., 1999) indicated that nesting may also induce a stress response in female olive ridleys. Circulating basal corticosterone was significantly elevated in a



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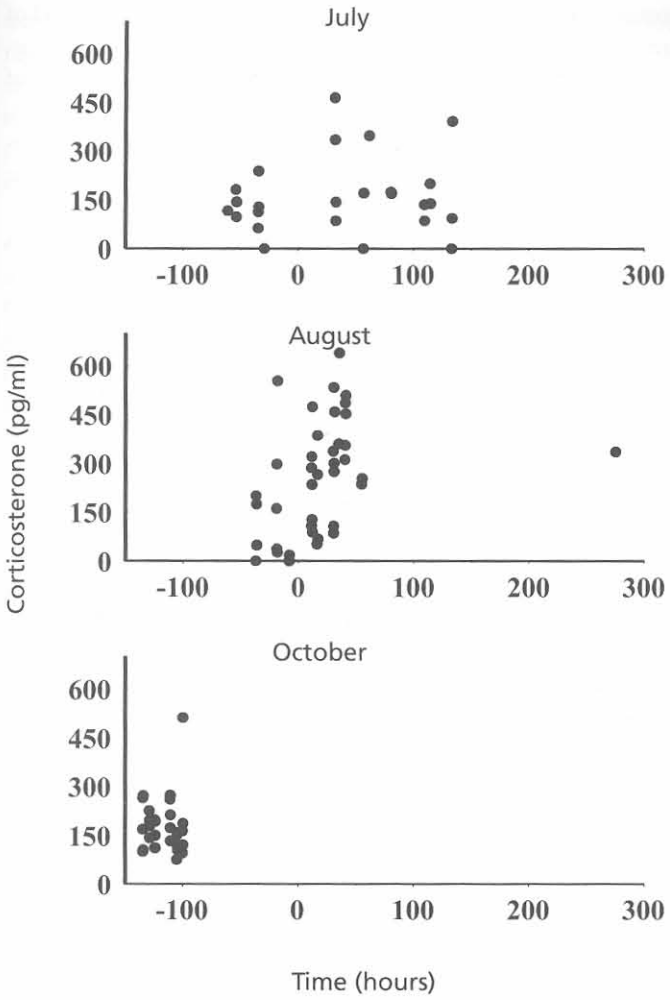

Fig. 7.2. Serum corticosterone levels in basking female olive ridley sea turtles captured throughout the day. Time 0 represents the onset of the *arribada* each month.

group of nesting arribada females randomly sampled at Nancite after completion of nesting, compared to basking females at the same location (Valverde et al., 1999). This may be attributed to AVT, a neurohormone secreted by the neurohypophysis, which has been shown to increase sharply in blood concentration in olive ridleys at the beginning of the nesting process, reaching a peak at the time of oviposition and returning to basal levels on the return of the turtles to the ocean (Figler et al., 1989). The role of AVT is presumably to regulate oviductal contractions by which eggs are forced out of the oviduct. It is possible that secretion of neurohypophyseal hormones such as AVT, which is also known to potentiate CRH-induced ACTH pituitary release in many vertebrate species (Fryer and Leung, 1982; Lilly et al., 1989; Harvey and Hall, 1990), may activate the pituitary-adrenal axis during nesting, resulting in the observed rise in corticosterone in nesting female turtles. If true, this evidence would support the idea that the ridley pituitary gland is sensitive to hypothalamic CRH during arribada nesting.

More recently juvenile Kemp's ridleys were shown to respond to the stress of capture and handling with increased corticosteroid output and increased blood glucose (Gregory and Schmid, 2001). In this study, wild juvenile Kemp's ridleys were captured by entanglement nets. Blood samples were taken at 0, 30, and 60 minutes after release from the nets. Both circulating

corticosterone and glucose concentrations increased over time following capture. The concentration of corticosterone at time zero in this study was 10-fold higher than basal levels measured in other studies of juvenile Kemp's ridleys and adult olive ridleys (Valverde et al., 1999; Ortiz et al., 2000). This suggests that concentrations observed at time zero after net capture were already well above basal values. The actual time that turtles spent in the net was not known, with a reported maximum possible time of 15 minutes before samples were taken. Because the HPA axis of other reptiles has been shown to be significantly activated within 10 minutes after capture (Moore et al., 1991; Dunlap and Wingfield, 1995), and the adrenal gland can respond more robustly to stimulation after ACTH priming by prior stress (Ehrhart-Bornstein et al., 1998), it is possible that the HPA axis of juvenile ridleys also was rapidly activated by capture stress.

A comprehensive study on the stress response of arribada olive ridleys at Nancite Beach, Costa Rica, was undertaken by Valverde (1996). During arribadas, females tend to concentrate in a relatively small area, often encountering many physical obstacles including their conspecifics and beach debris. In spite of such high interaction frequency, turtles complete their nesting successfully. This has led to the hypothesis that arribada ridleys might have evolved a central mechanism that increases their sensory threshold, resulting in a lower response to environmental stimulation (Valverde, 1996; Valverde et al., 1999). This hypothesis predicted that nesting arribada turtles would exhibit a hyporesponsive HPA axis. To test the hypothesis, nesting arribada turtles were captured and turned on their backs for blood sampling over the subsequent 6 hours, after the completion of egg laying. Solitary nesting and basking females, as well as males, were also captured as controls and subjected to the same restraint and sampling protocol. The "turning stress" was very instrumental in the stimulation of the ridley HPA axis; turtle groups exhibited a corticosterone response to turning. However, males responded more rapidly (significant increase over basal levels by 20 minutes) than any female group, with the arribada turtles responding at the slowest rate (significant increase by 120 minutes). By the end of the 6 hours, all groups had reached similar circulating mean corticosterone concentration of approximately 4 ng/ml (Valverde et al., 1999). These data support the hypothesis that the arribada females undergo an inhibition of their HPA axis.

Two aspects of interest in this study are that solitary turtles tended to exhibit a slightly higher adrenal response than arribada and basking turtles, indicating that solitary turtles were more sensitive to the physical stressor. The other interesting aspect is that a subgroup of the female turtles in every group studied exhibited no detectable responsiveness to turning stress (refractory females) (Valverde et al., 1999). The former data suggest the interesting possibility that solitary turtles may be too sensitive to physical stimulation to nest at the high densities that characterize arribada events. The latter data suggest that a subpopulation of nesting females at Nancite is experiencing active inhibition of their HPA axis, an inhibition that was not observed in any male olive ridley. A caveat in the latter observation is that the males included in the study were mating at the time of capture. It has been shown that mating olive ridley males exhibit higher circulating corticosterone concentration than mating females (Schwantes, 1986). It is therefore possible that the sensory systems of mating males must remain alert to environmental stimulation because other males may try to dislodge them during the mating process (Booth and Peters, 1972; Alvarado and Figueroa, 1989). Nevertheless, in general these results support the hypothesis that inhibition of the arribada female HPA axis allows them to complete the nesting process in spite of extensive physical contact with and disturbance from conspecifics during arribadas.

An important question related to this hypothesis is whether the sea turtle HPA axis exhibits periods of higher and lower activity during key life history events, such as reproductive and migratory activities, as has been amply demonstrated in birds (the so-called "adrenal modulation," e.g., Wingfield and Kitaysky, 2002). To this effect it has been shown that female green and hawksbill sea turtles exhibit a depressed adrenal response to a physical stressor during reproduc-

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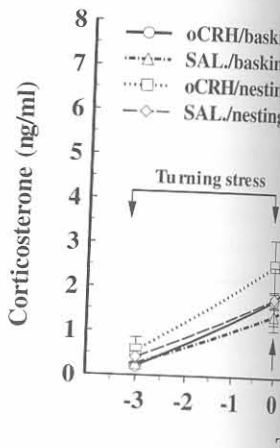

tive periods with respect to animals that are not reproductively active (Jessop, 2001). It has been suggested that this reduced adrenal response may play an important role in ensuring that reproduction will indeed carry on in spite of environmental stressors (Wingfield et al., 1998).

In an attempt to identify possible mechanisms responsible for the inhibition of the HPA axis in arribada females, several experiments were performed. To test the hypothesis that a steroid hormone from the developed ovary suppresses the HPA axis, circulating testosterone concentrations were measured in the same turtles (Valverde et al., 1999). Testosterone production in female ridleys has been shown to be maximal in fully developed ovaries and minimal in the quiescent ovary (Rostal et al., 1997, 1998). Testosterone concentration did not differ between responsive and refractory females, indicating that refractoriness was not related to ovarian testosterone production or to clutch number.

To test the hypothesis that ridley pituitary sensitivity was suppressed, basking and nesting turtles were subjected to turning for 3 hours (to identify potentially refractory females) (Valverde, 1996) and then injected into the cervical sinus with an approximate average dose of 1.22 μg ovine CRH/kg body mass; control turtles received saline. Blood samples were obtained 0, 2, and 6 hours postinjection. Adrenal response showed that all animals responded to turning stress with increased blood corticosterone (Fig. 7.3). Although this confirms the functionality of the ridley HPA axis, the lack of refractory females precluded a full testing of the hypothesis. However, these data do suggest a lack of sensitivity of the HPA axis to ovine CRH, but it is possible also that the CRH did not reach the pituitary or that the system was already maximally stimulated by the turning stress alone. The lack of sensitivity of ridleys to CRH would be in contrast to the human pituitary, which is capable of responding to intravenous CRH injections in a dose as low as 0.03 μg/kg body mass (Coiro et al., 1995). Further study, ideally utilizing a dose-response approach, is required to address the issue of the role of CRH in regulating ridley ACTH secretion.

Finally, to test the hypothesis that the adrenal gland was refractory to ACTH stimulation in nesting olive ridleys, arribada females were first subjected to turning stress for 4 hours, then injected with either porcine ACTH (0.6 IU pACTH/kg of body mass) or saline solution. Turtles were held in a special corral for 24 hours postinjection in which they were able to move freely. Results showed that all turtles responded to turning stress with equivalent increased blood corticosterone, again precluding testing the refractory HPA axis hypothesis. However, ACTH-injected females did show a significant increase in corticosterone with respect to controls at all times after injection. Thus, although hypothalamic control of pituitary ACTH has yet to be established, the ridley adrenal gland is capable of rapid activation by exogenous ACTH of greater magnitude than elicited by turning stress. This supports the suggestions that the ridley pituitary is

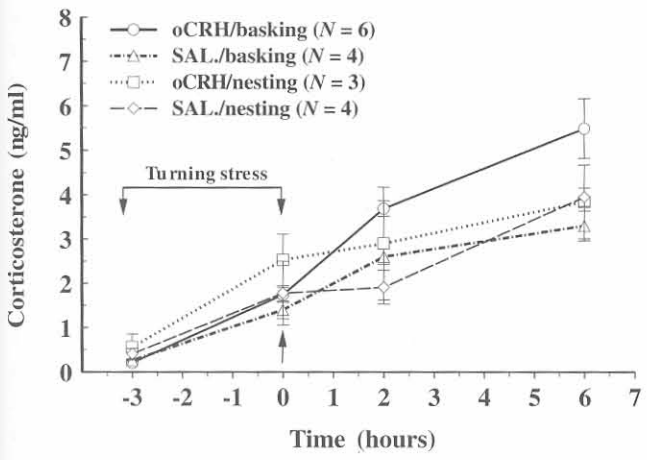

Fig. 7.3. Adrenal response to turning and ovine corticotropin-releasing hormone (CRH) or saline injections in nesting and basking olive ridley sea turtles. At time 3 hours, animals were turned on their backs; at time 0, animals were turned on their plastrons, injected in the cervical sinus, and then allowed to wander in an enclosure for the remainder of the experiment. Figure shows mean corticosterone values ± SEM. Single arrow indicates time of injection.

not producing enough ACTH to effectively control the adrenal gland and that the HPA axis is centrally inhibited during nesting.

From the previous discussion, at least two experiments are suggested. First, it would be necessary to test the hypothesis that only breeding olive ridleys undergo inhibition of their HPA axis, as has been shown for green turtles (Jessop, 2001). This hypothesis was addressed by searching for olive ridleys in international waters of the eastern tropical Pacific Ocean, away from nesting grounds (Owens, 1993), to capture only reproductively inactive adult olive ridleys (based on blood testosterone concentrations <20 pg/ml) (Plotkin et al., 1995). Two mature males and one female (testosterone = 35 pg/ml with small developing follicles) were captured and subjected to turning stress, with blood sampling for a period of 6 hours (Fig. 7.4). Males were further subjected to laparoscopy for gonadal examination and subsequently blood-sampled. Data showed that all animals responded to capture and turning stress with increased blood corticosterone, in a manner similar to animals near or at the nesting beach (Valverde, 1996). Males responded with higher production of corticosterone than the female, but the small sample size precluded statistical analysis. Thus, it was not possible to test adequately the hypothesis that reproductively quiescent turtles would exhibit a more robust stress response. Further work in this area is needed.

The second suggested experiment would be important to determine whether only arribada species, which are subjected to much greater disturbance on nesting beaches, exhibit diminished adrenal responsiveness. To address this hypothesis, nesting loggerhead turtles were also subjected to turning stress and compared with solitary nesting olive ridleys (Valverde, 1996; Valverde et al., 1996). If HPA axis inhibition was exclusive to ridleys, loggerheads (a solitary nesting species) should exhibit a robust response to turning. However, loggerheads not only responded sluggishly to the turning stress, as do olive ridleys, but also exhibited a lower magnitude of response than the solitary olive ridleys by the end of the experiment (Fig. 7.5). Interestingly, it has been shown that green sea turtles do not exhibit increased adrenal output in response to increased nesting density (Jessop et al., 1999). This suggests that green turtles may also be hyporesponsive to physical stimulation during nesting. Thus, the hypothesis of the exclusiveness of the hypoactive ridley HPA axis is not supported.

The data discussed up to now suggest that the HPA axis of the olive ridley, and perhaps that of sea turtles in general, responds sluggishly to physical stress. This is particularly clear in com-

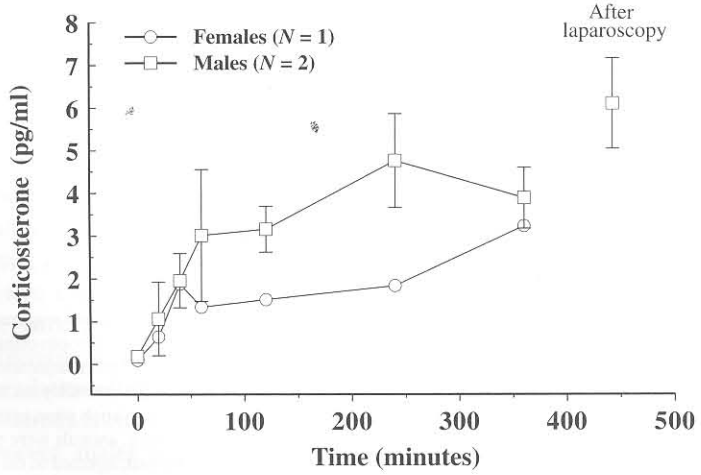

Fig. 7.4. Mean circulating corticosterone levels (± SEM) in two male and one female olive ridleys captured in the open ocean and subjected to turning stress. Last mean value for males represents corticosterone levels measured in samples obtained after laparoscopic surgery.

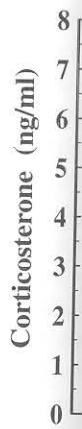

Fig. 7.5. Mean circulating cort
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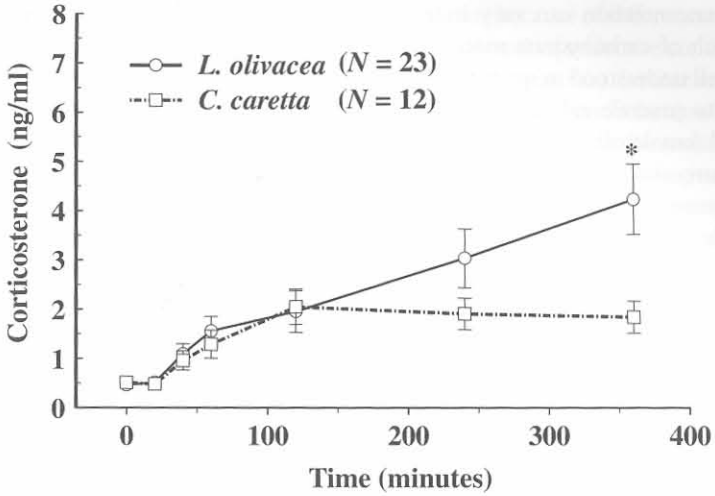

Fig. 7.5. Mean circulating corticosterone levels (± SEM) in nesting female olive ridley and loggerhead sea turtles subjected to turning stress. Asterisk indicates significant differences between mean corticosterone levels of loggerhead and olive ridley females at 360 minutes from initiation of the experiment ($P = 0.008$).

parison to other vertebrates, such as birds, fish, and lizards, that exhibit significant elevations within 10 minutes in response to capture and handling (Dunlap and Wingfield, 1995; Kakizawa et al., 1995; Wada and Shimizu, 2004). The ridley adrenal gland, however, does not appear to be insensitive to stimulation, suggesting that the sluggishness of the system is likely the result of a central mechanism that operates to increase sensory thresholds of the turtles during nesting. Recently, an evolutionarily ancient CRH binding protein was detected in the hypothalamus of the turtle (Seasholtz et al., 2002). This protein has been characterized in vertebrates as a high-affinity binding protein, capable of modulating the bioactivity of CRH and, presumably, the activity of the HPA axis. It is possible that the production of this protein is elevated during reproduction in the female turtle, which would in turn prevent fast activation of the HPA axis. If supported, this mechanism may be linked to the high disturbance threshold (HDT), a trance-like behavior that all nesting sea turtle species exhibit, particularly during egg laying (Valverde, 1996). This HDT behavior has been described in the literature as a period when turtles appear to be quiescent, oblivious to physical (visual, auditory, tactile) stimulation (Hughes and Richard, 1974; Ehrenfeld, 1979). Such a common link could explain why the HPA axis of the loggerhead sea turtle may respond similarly to that of the olive ridley.

GLUCOSE AND STRESS. Blood glucose concentration has also been used to assess the impact of a stressor on the systemic homeostasis of the organism (Widmaier, 1990; Chrousos and Gold, 1992). Thus, circulating glucose measurements may be used as a diagnostic tool for stress in ridleys. Indeed, wild juvenile Kemp's ridleys have been reported to respond to capture and handling with elevated blood glucose within 1 hour of capture (Gregory and Schmid, 2001), indicating that ridleys can exhibit a hyperglycemic response to a stressor. However, it is not known whether glucose increases are caused by adrenocorticosteroid activity or another factor, such as catecholamine hormones. Interestingly, blood glucose has been shown to remain unchanged in the face of variations in the external osmotic environment in captive juvenile Kemp's ridley sea turtles (Ortiz et al., 2000). This, and the lack of adrenal responsiveness to osmotic changes, suggest that the external osmotic environment does not represent a stressor to the ridley's physiology. However, it is important to keep in mind

that glucose concentration can vary independently as a result of carbohydrate metabolism, which is not well understood in sea turtles.

In contrast to juvenile ridleys, free-ranging adult male and female olive ridleys did not respond to capture and turning stress with increased concentration of blood glucose within 6 hours (Valverde et al., 1999). In a subsequent study, arribada olive ridleys subjected to turning stress for 4 hours exhibited basal glucose levels similar to those observed in the 6-hour study (Valverde, 1996). However, when these animals were injected with saline or ACTH and subsequently held in captivity on land for a period of 24 hours, blood glucose dropped significantly to about half initial levels. Thus, the results of these two studies do not support a hyperglycemic role for glucocorticoids in reproductively active male and female olive ridleys. An interesting aspect of this study is that basal blood glucose concentrations of these turtles were significantly below those of turtles captured in feeding areas for the population in the eastern tropical Pacific Ocean. It has been suggested that sea turtles do not feed actively during the reproductive months (Owens, 1976, 1980). Evidence for this hypothesis has recently been reported (Tucker and Read, 2001). It is possible that this hypophagia might limit the energy stores of the turtle. This energy limitation may in turn impact not only the gluconeogenic capacity of glucocorticoids by decreasing the availability of noncarbohydrate substrates but also the hyperglycemic effect of other stress hormones such as catecholamines. From the above discussion, it seems that the use of blood glucose is not a reliable tool to describe the stress response of the actively reproductive ridley turtle.

Salinity and Adrenocortical Response

Ridleys, like all other sea turtles, live in a hyperosmotic environment. In order to help maintain ion homeostasis, these marine reptiles have evolved a highly efficient salt gland (Reina et al., 2002). Secretory activity of the sea turtle salt gland is thought to be partially under the control of adrenal steroids (Holmes and McBean, 1964). Using captive-raised Kemp's ridleys, Morris (1982) studied adrenal responsiveness to a change in ambient salinity in the context of salt gland function. In a series of experiments, 3-year-old Kemp's ridleys were subjected to changes in salinity. Turtles were transferred from salt water at 34 parts per thousand (ppt) to either 34 ppt (control), 17 ppt, or 0 ppt salinity. An hour after transfer, all treatment groups showed significant, equivalent elevations in circulating corticosterone (up to a mean of approximately 9 ng/ml), which then declined to basal levels (less than 2 ng/ml) by 6 hours. Two weeks after transfer, all turtles were transferred back to 34 ppt. Following this second transfer, no increases in corticosterone were observed at 5 or 24 hours, although, based on results of the first experiment, this sampling interval would have missed acute corticosterone changes in the first hour. In a second experiment, Kemp's ridleys were intraperitoneally injected with 0.25 ml/kg of either 30% or 0.9% (physiological) saline. Both groups of turtles exhibited elevated corticosterone by 30 and 60 minutes, but no differences were found between the two treatments. Although these two sets of experiments do suggest activation of the adrenal axis with handling or sampling, as has been shown more recently for captive Kemp's ridleys (Stephenson et al., 2000), they do not support a direct role of adrenal glucocorticoids in the adaptation to osmotic challenges. To our knowledge, the studies by Morris (1982) were the first to demonstrate that the ridley HPA axis can respond to physical stressors with robust production of corticosterone.

In a similar study, Ortiz et al. (2000) examined water flux and osmotic and adrenal responses to acute salinity challenge in four juvenile, captive-raised Kemp's ridleys. Full-strength seawater-acclimated animals were switched to fresh water for 4 days and then back to full strength for another 7 days. Rapid water changes (<6 minutes) in the holding tank were achieved without handling animals, and blood samples were taken at 2-day intervals for measurement of aldosterone and corticosterone. No changes were found in either hormone following transfers in spite of significant changes in water flux, plasma ion composition, and plasma osmolality. Although these data suggest that these steroids do not play an important role in the osmoregulatory response of ridley turtles, it is again possible that

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Catecholamine Hormo
and Stress

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more frequent sampling might have revealed transient hormone changes. Both Morris (1982) and Ortiz et al. (2000) suggest that acute changes in ambient salinity do not constitute a stressor to Kemp's ridleys.

Catecholamine Hormones and Stress

Chromaffin cells of the adrenal medulla, a peripheral component of the sympathetic nervous system, release the catecholamine hormones epinephrine and norepinephrine (also known as adrenaline and noradrenaline). In mammals, catecholamine hormones induce rapid energy mobilization as well as increased cardiac output and blood pressure, which are thought to mediate adaptation to acute stress (Chrousos and Gold, 1992). Studies in freshwater turtles have found a significant (less than 1 hour) elevation of blood epinephrine, norepinephrine, and glucose during diving hypoxia (e.g., Wasser and Jackson, 1991), consistent with a role for these hormones in glucose mobilization. The only report on ridley catecholamine hormones is that of Hoopes et al. (2000). This study has been described in more detail in the previous section on Entanglement Net Capture Stress. Wild, juvenile Kemp's ridleys were retrieved after spending less than 10 minutes in entanglement nets, and blood was sampled for up to 48 hours while they were held in in-water cages and on-shore tanks. Blood concentrations of both epinephrine and norepinephrine were elevated at the initial sample and then declined over 6–10 hours, reaching minimal levels at 48 hours. Hoopes et al. (2000) suggest that postcapture holding conditions influenced the rate of recovery from stress because catecholamine levels declined more rapidly in turtles held in in-water cages than in turtles held in on-land tanks. The only other study of catecholamine hormones in sea turtles found no change in circulating epinephrine and norepinephrine in postnesting female green turtles restrained for up to 10 minutes (Hamann et al., 2003). Hamann et al. (2003) suggest that this lack of elevation may be a result of desensitization of catecholamine production during nesting, similar to the situation discussed above for glucocorticoids. Although these two studies are difficult to compare because of differences in capture protocols, age, and reproductive condition of the turtles, both suggest that further study of the dynamics of blood catecholamine hormones will provide information on the sensitivity of sea turtles to natural and human-imposed stressors. It will be challenging to conduct such studies in wild ridley turtles. To characterize the activation of catecholamine release, initial blood samples should be obtained from undisturbed animals. Furthermore, repetitive sampling to characterize recovery will require restraint of animals, for possibly as long as 24–48 hours.

Summary

Current available data indicate that ridley turtles possess a functional HPA axis. Moreover, the axis has been shown to function much like that of other vertebrate species. Specifically, the axis is activated by physical stressors and by pharmacological manipulation. Interestingly, the ridley HPA axis of the adult turtle exhibits a higher activation threshold than that of many other reptiles, and that of vertebrates in general, in response to physical stimulation. In addition, some ridley turtles can exhibit nearly complete endocrine (corticosterone) refractoriness to physical stressors. These aspects of the ridley HPA axis may assist the animals during nesting, particularly at high densities. The nature of this HPA axis modulation is not understood and warrants further investigation. In order to establish whether this phenomenon is related to reproductive condition or energy stores, it is important to conduct experiments with smaller ridleys located in the feeding grounds. The modulatory phenomenon of the ridley HPA axis suggests the possibility that these animals possess alternative homeostatic mechanisms that allow them to withstand the disruptive effects of stressors. To understand the role of glucocorticoids in the physiology of the ridley, the molecular and biochemical characterization of the ridley glucocorticoid receptor would be most informative. In addition, the evidence available on blood glucose supports the idea that the adult ridley effectively undergoes a period of hypophagia. This phenomenon seems to be restricted to the reproductive months and may impinge on

the ability of glucocorticoids and other hyperglycemic hormones to promote an elevation in blood glucose. More studies are required to elucidate the relationship between energy balance and the dynamic nature of the physiology of the HPA axis.

Thyroid Physiology

The regulation of thyroid hormone secretion and the actions of thyroid hormones at target tissues are not well understood for most commonly studied reptilian species. Because thyroid hormones consistently have been implicated as playing a role in supporting energetically demanding processes such as nutrient assimilation, growth, development, reproduction, and migration (Eales, 1979; McNabb, 1992), thyroid function in reptiles is of basic interest because of their evolutionary position at the transition to endothermy. More recently, reptilian thyroid glands have been studied because of the possibility of environmentally induced endocrine disruption (Crain et al., 1998). Studies of thyroid function in ridley turtles may thus contribute both to our basic understanding of the evolution of thyroid function and to a more practical understanding of the potential impact of anthropogenic chemicals on threatened species.

Studies in diverse vertebrate species have led to a consistent picture of the basic organization of the hypothalamo-pituitary-thyroid axis (Fig. 7.1; McNabb, 1992; Hulbert 2000): hypothalamic peptide hormones regulate secretion of pituitary TSH; TSH in turn activates the synthesis and secretion of the thyroid hormones thyroxine (tetraiodothyronine or T_4) and 3,3′,5-triiodothyronine (T_3) from the thyroid gland. TSH stimulates unique processes in thyroid cells, including iodide uptake and organification, thyroglobulin synthesis, thyroid hormone synthesis and cleavage from thyroglobulin, and thyroid hormone secretion. TSH also promotes thyroid cell growth and differentiation. In most vertebrates examined, T_4 is the predominant hormone released to the circulation from the thyroid gland (McNabb, 1992).

Data available for ridley turtles address relatively few aspects of thyroid function. Indeed, the first comprehensive review of reptilian thyroid function (Lynn, 1970) makes only a single reference to the genus *Lepidochelys*. This is a description of the histological appearance of the thyroid gland of the olive ridley (Yamamoto, 1960), which demonstrates the characteristic follicular appearance consistent with the general vertebrate structure supporting thyroid hormone synthesis. More recent studies on circulating thyroid hormones, described below, lead us to believe that thyroid function in *Lepidochelys* resembles the generalized vertebrate model. Additionally, comparative studies have confirmed the existence of the basic elements of the hypothalamic-pituitary-thyroid axis in turtles. A TSH homologous to that found in other vertebrates has been purified from the green turtle (MacKenzie et al., 1981), and we expect that a similar protein exists in ridley pituitaries. This TSH has been used for the development of an RIA capable of measuring TSH in blood and pituitary tissue of freshwater and marine turtles. Use of the RIA has confirmed that thyroid hormones exert a negative feedback on pituitary TSH production in green turtles (MacKenzie et al., 1981) and in slider turtles (Denver and Licht, 1988). It has also identified several hypothalamic peptides, including thyrotropin-releasing hormone (TRH) and others conventionally associated with the regulation of pituitary hormones such as GH or ACTH, capable of stimulating pituitary TSH secretion in slider turtles (Denver and Licht, 1989, 1990). The ability of CRH to stimulate TSH release in slider turtles suggests a linkage between the thyroid and adrenal systems (Denver and Licht, 1989). Further study of hypothalamic regulation of TSH in sea turtles would thus provide a broader comparative perspective on the regulation of TSH secretion and a better understanding of their integrated endocrine response to stress. Presently, no information is available on the nature of TSH in ridley turtles or its hypothalamic control.

The slider turtle thyroid axis is now the most intensively studied of any reptilian species. In contrast, the limited availability of sea turtle species for experimental or descriptive examination of thyroid function has hindered our understanding of how applicable slider turtle data are to nonemydid turtles. In the case of thyroid hormone blood transport, for example, emydid

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Table 7.2 C
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Species
Lepidochelys kemp
Caretta caretta
Chelonia mydas
Trachemys scripta
Homo sapiens

Note: Data from Ha
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turtle serum.

[a]Affinity units: 10^7

[b]Capacity units: 10

turtles appear quite unusual among ectothermic vertebrates. Thyroid hormones circulate in the blood bound to several binding proteins that serve to facilitate their transport to distant tissues (Fig. 7.1). In slider turtles, a unique vitamin D binding protein binds T_4 with high affinity in the circulation (Licht, 1994). Circulating T_4 stimulates the production of this binding protein, resulting in periods of the year when blood binding capacity, and therefore circulating T_4, is among the highest observed in any vertebrate, including mammals (Licht et al., 1990). Phylogenetic studies of the distribution of this binding protein in reptiles show that it is not present in nonemydid species, including olive ridley (Licht et al., 1991). This was confirmed by Haynes (1990) in a detailed analysis of thyroid hormone binding to sea turtle serum proteins. In comparison to humans and *Trachemys*, serum from sea turtles, including Kemp's ridley, showed a diminished ability to bind both T_3 and T_4. Scatchard analysis of binding to Kemp's ridley serum demonstrated the presence of a single, high-affinity, moderate-capacity T_4 binding site with an affinity similar to the human thyroid hormone transporter transthyretin (Table 7.2). Binding affinity of T_3 in Kemp's ridley blood was about 10-fold lower than that of T_4. This study concluded that Kemp's ridley turtles possess a single, moderate-affinity thyroid hormone transport protein in serum. Phylogenetic studies of transthyretin (Power et al., 2000) have indicated that it is not present in reptile blood, suggesting that a different, unknown protein functions as the primary thyroid hormone transport protein in ridley turtles. Further studies are needed to determine whether modulation of the blood content of this protein serves to alter thyroid hormone delivery to peripheral tissues.

At peripheral tissues, T_4 enters target cells and is converted to deiodinated metabolites by intracellular deiodinase enzymes (Fig. 7.1). Outer ring deiodinases (ORD) convert T_4 to T_3, which, because it generally has a higher affinity for the nuclear thyroid hormone receptor, is considered the active intracellular form of thyroid hormone (McNabb, 1992; Hulbert, 2000). Inner ring deiodinases (IRD) can also convert T_4 to a variety of deiodinated metabolites, many of which are considered to be inactive breakdown products of thyroid hormone (Hulbert, 2000). The T_3 generated by ORD can be made available to nuclear thyroid hormone receptors to regulate the transcription of specific genes, can be broken down

Table 7.2 Characteristics of thyroid hormone binding to serum proteins in sea turtles

	T_3			T_4		
Species	Site	Affinity[a]	Capacity[b]	Site	Affinity[a]	Capacity[b]
Lepidochelys kempii	1	0.855	2.43	1	6.29	2.48
Caretta caretta	1	0.554	1.96	1	7.81	0.604
Chelonia mydas	1	1.20	2.17	1	4.89	4.73
Trachemys scripta	1	1.614	0.144	1	83.9	0.188
Homo sapiens	1	40.91	0.096	1	255.7	0.0484
	2	1.79	0.117	2	8.43	0.665

Note: Data from Haynes (1990). Serum samples were pooled, and endogenous thyroid hormone was removed using ion-exchange resin. Serum was then subjected to saturation analysis using radioiodinated thyroid hormones on Sephadex minicolumns. All assays were performed at 20°C. Data were analyzed using the LIGAND computer program to determine the number of thyroid hormone binding sites in each species, the affinity of each binding site for thyroid hormones, and the total binding capacity of the serum for each thyroid hormone. Data for *T. scripta* and *H. sapiens* are included for comparison to sea turtle serum.

[a]Affinity units: 10^7 M^{-1}.

[b]Capacity units: 10^{-6} M.

further intracellularly to inactive products, or can be released back to the blood, in considerable amounts in some species, where it presumably is capable of activating additional thyroid hormone receptors at distant targets (Fig. 7.1; Eales and Brown, 1993). Eventually, thyroid hormone metabolites are conjugated and excreted by the kidney and the liver, although evidence exists in some species for an active enterohepatic cycling system that may return significant amounts of thyroid hormone to the circulation through deconjugation by bacterial flora in the gut (Eales and Brown, 1993). We have no information for any sea turtle species on any of these peripheral processes critical to the activation and reception of thyroid hormones at their target tissues. The threatened/endangered status of ridley turtles has precluded directed or opportunistic collection of fresh tissue needed for in vitro enzyme and receptor assays. However, molecular biological techniques now permit characterization of the expression of the genes for these proteins in minute tissue samples and could be applied to ridley turtle tissue to confirm the existence of deiodination enzymes and thyroid hormone receptors.

Currently, the most commonly used method for evaluation of thyroid function in reptiles is measurement of circulating thyroid hormone concentrations. This can be achieved relatively easily through the collection of blood samples, followed by measurement of thyroid hormones using a validated RIA. Although it gives a static picture of the total thyroid hormone concentration in blood at a particular point in time, this technique provides little information on the nature of the binding of thyroid hormones to blood proteins or to the actual rate of delivery of thyroid hormones to receptors in target tissues. Nonetheless, this approach is useful in identifying periods of hypothalamic-pituitary-thyroid axis activation, potentially representing those times at which thyroid hormone secretion or hormone stimulation of target tissues is greatest. This is normally the first step in initiating a more detailed examination of thyroid function. For example, a dramatic midsummer peak in T_4 in slider turtles (Licht et al., 1985) suggested that environmental temperature may activate the thyroid axis and that thyroid hormones may be involved in somatic growth, both later confirmed by laboratory experimentation (Licht et al., 1989; Denver and Licht, 1991). The power of hormone measurement is enhanced with an increased frequency of sampling, giving a more precise picture of the dynamics of activation of the pituitary-thyroid axis. A common criticism of blood measurement studies is that sampling at one time of day may fail to detect dynamic circadian changes in blood thyroid hormones, as has been noted for a number of fish species (Leatherland, 1994). In this regard, it is important to note that Moon et al. (1999) measured circulating T_4 in seven blood samples taken over 48 hours from captive Kemp's ridleys, held at constant temperature and light cycle, and detected no evidence of a daily fluctuation. However, thyroid hormones might not be as stable in the wild, under conditions of variable temperature, photoperiod, and feeding. Channel catfish, which exhibit no daily changes in thyroid hormones when held in the laboratory (Gaylord et al., 2001), display dramatic increases in both T_3 and T_4 in the afternoon when held in outdoor ponds (Loter, 1998).

Several studies have described circulating thyroid hormones in ridley turtles (Owens, 1997). Moon et al. (1998) have provided the only data on T_4 in wild ridley turtles, reporting a mean of 6.7 ng/ml for nesting and 3.3 ng/ml for swimming olive ridleys in Mexico. Although samples can be collected easily during the brief period of nesting in females, this limited perspective on thyroid hormones in wild turtles is of minimal value without comparative data from other times or physiological conditions. Our most detailed information on seasonal thyroid hormones in ridley turtles comes from captive animals. Moon et al. (1998) found seasonal changes in circulating T_4 in adult female, but not adult male, Kemp's ridleys held in indoor tanks under artificial simulated natural photoperiod and natural temperature in Galveston, Texas. In females, the distinct annual cycle showed elevated T_4 in March to May, as water temperatures were increasing, followed by a decline in July to October, when temperatures were maximal. A second peak in T_4 occurred in late November to December. Mean T_4 in both sexes ranged from 5 to 13 ng/ml, substantially lower than that in

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slider turtles but at the higher end of the range reported for most wild reptiles (e.g., Bona-Gallo et al., 1980; John-Alder, 1984; Naulleau et al., 1987; Kohel et al., 2001). Rostal et al. (1998) also found seasonal changes in a captive population of Kemp's ridleys held in large outdoor ponds under natural photoperiod and temperature at the Cayman Turtle Farm. Although the history and environmental conditions were quite different for this group of animals, the range of T_4 was similar to that in the Galveston ridleys. Once again, males exhibited a low magnitude of T_4 changes with the exception of a significant elevation of T_4 (over 5 ng/ml) found only in March. Females showed more dynamic annual T_4 changes, with a major T_4 peak (near 11 ng/ml) in December, followed by a smaller peak in March and a gradual decline in summer.

The sexual dimorphism in T_4 cycles at both locations suggests an interaction between reproductive condition and thyroid function. A reciprocal relationship between T_4 and testosterone, noted in prior reptilian studies (Bona-Gallo et al., 1980; Licht et al., 1985; Naulleau et al., 1987), was not apparent in Kemp's ridleys. It is possible that the relatively low sampling frequency (once every 1–3 months) may have missed some transient T_4 elevations, yet similarities between the two locations are intriguing. In both groups of females, maximal T_4 was observed at the time of initiation of ovarian recrudescence. This coincided with elevated blood vitellogenin (as indicated by blood calcium) (Rostal et al., 1998). A second T_4 elevation coincided with mating activity. The consistent results between these two captive populations suggest that T_4 participates in the energetically demanding process of vitellogenesis, possibly promoting the mobilization of lipid and protein in the liver for vitellogenin synthesis. Alternatively, changes in blood composition associated with vitellogenesis may alter T_4 transport by increasing thyroid hormone binding to blood proteins. However, Heck et al. (1997) did not find support for binding of T_3 or T_4 to vitellogenin in Kemp's ridleys; pharmacologically elevated blood vitellogenin had no effect on plasma thyroid hormone binding up to 75 days following estrogen treatment. Elevated T_4 during vitellogenesis, whether associated with vitellogenin or bound to other blood proteins, raises the possibility that it can move into the yolk of ridley eggs, as has been noted for avian and teleost species (Wilson and McNabb, 1997; Tagawa and Brown, 2001). Additional studies of thyroid hormone transport and incorporation into yolk are needed to establish a role for maternal hormones in the regulation of sea turtle embryogenesis. If thyroid hormones are incorporated into ridley eggs and play a significant role in the regulation of embryonic development, as has been proposed for slider turtle steroid hormones (Bowden et al., 2002), disruption of maternal thyroid function in wild populations may influence embryonic development. Such disruption might include alteration of maternal thyroid hormone secretion, deiodination, blood transport, or receptor binding (Brucker-Davis, 1998; Eales et al., 1999). Exposure of marine birds to aromatic hydrocarbons has been found to alter thyroid mass, circulating thyroid hormone levels, and egg yolk composition (Rolland, 2000), suggesting that animals living in contaminated marine habitats may be vulnerable to endocrine disruption.

Blood T_3 in captive or wild ridleys is normally at or below the sensitivity of the RIAs used (<0.1 ng/ml) (Rostal et al., 1998; Moon et al., 1998, 1999). Low or nondetectable T_3 has been noted in a number of reptilian species (John-Alder, 1984; Licht et al., 1990; Kohel et al., 2001). Circulating T_3 reflects a combination of factors, including rates of formation, blood protein binding, and clearance (McNabb, 1992). Active deiodinases have been characterized in a variety of tissues from slider turtles (Hugenberger and Licht, 1999), a species with similar nondetectable T_3, demonstrating that low T_3 does not necessarily reflect a lack of peripheral deiodination. Low blood T_3 in Kemp's ridleys is likely caused in large part by a low affinity of T_3 binding to serum proteins (Table 7.2). In this regard, ridley turtles resemble mammals, which exhibit similar low circulating T_3 in spite of active outer ring deiodination in many peripheral tissues. Barely detectable T_3 (up to 0.9 ng/ml) has been noted in some captive Kemp's ridleys maintained on constant warm temperatures and daily feeding of a high-protein diet (Moon et al., 1998, 1999). However, additional T_3 data from wild animals

under a variety of physiological conditions are clearly needed for a better understanding of the significance of circulating T_3.

Seasonal T_4 cycles in ridley turtles are likely a result of multiple influences. One variable that may seasonally modulate thyroid hormone secretion and transport is body temperature (Licht et al., 1989). To determine if temperature influences blood thyroid hormones in Kemp's ridleys, Moon et al. (1997) subjected a group of 10 immature Kemp's ridleys to a controlled decrease in temperature. Although animals remained active as temperature dropped from 25°C to 10°C, time of submergence increased and food consumption decreased below 15°C. A dramatic decline in circulating T_4 also was observed at all temperatures below 25°C (Moon, 1992). Because studies of slider turtles have shown that alterations in thyroid status or environmental conditions that diminish blood binding protein capacity result in substantial reductions in circulating thyroid hormone concentrations (Licht et al., 1990), we evaluated these Kemp's ridley blood samples for thyroid hormone binding to serum proteins at the acclimation temperatures of the turtles, using a Sephadex column index of total thyroid hormone binding (Haynes, 1990). In Kemp's ridley serum pooled from animals acclimated to 30°C, both T_3 and T_4 binding were relatively stable over the range of temperatures from 10°C to 40°C. In contrast, the blood samples taken from turtles acclimated to low temperatures showed significantly lower binding of T_4, but not T_3, at 15°C (Haynes, 1990). These studies support a role for thyroid hormones in promoting energy utilization during periods of elevated temperature or increased activity in Kemp's ridleys. They also indicate that decreases in body temperature may reduce thyroid hormone delivery to target tissues (Haynes and MacKenzie, 1990). Alterations in thyroid hormone delivery may thus be one facet of the deleterious changes that contribute to physiological impairment associated with cold temperature (e.g., cold stunning) (Milton and Lutz, 2003).

Because turtles that are cold also cease feeding, Moon et al. (1999) evaluated the possibility that the decline of circulating thyroid hormones might be caused in part by reduced food intake. However, no changes in T_4 or T_3 were observed in captive Kemp's ridleys during 2 weeks of food deprivation. In a reciprocal experiment, in which feeding was increased 250%, satiated turtles also showed no significant changes in T_4 or T_3 in comparison to control (fed) turtles. The lack of response to alterations in ration may be a species-specific phenomenon in ridley turtles because captive green sea turtles in the same experiment did exhibit significant changes in both T_3 and T_4. The thyroid response to food intake is complex, and previous nutritional history may influence the magnitude of response to food deprivation (MacKenzie et al., 1998). Kemp's ridley studies do not provide support for the activation of thyroid hormone production by nutrient intake, as has been suggested for other vertebrates (MacKenzie et al., 1998). However, well-fed captive animals maintain significant nutritional stores, particularly fat (Owens, 1997), which may minimize the effects of this relatively brief food deprivation. Again, studies of thyroid function in wild animals undergoing natural cycles of food intake may help elucidate the significance of these findings.

Summary

The limited data available suggest that ridley turtles have a thyroid gland capable of increased T_4 production during times of anabolic activation. Reproductive activity and increased temperature may both enhance thyroid hormone secretion and transport. Distinct annual T_4 cycles have only been observed in captive female Kemp's ridleys; further clarification of the role of thyroid hormones in the physiology of ridley turtles must await a more detailed evaluation of wild animals. In addition, more information is needed on the dynamics of thyroid hormone secretion to determine whether relatively infrequent sampling protocols, often an unavoidable limitation in sea turtle studies, provide representative data. Because of the stability of thyroid hormones in well-preserved serum, blood samples already stored in freezers could be analyzed relatively easily for T_3 and T_4. Such studies would provide us with needed baseline data on the normal range of thyroid hormones under diverse physiological and environmental condi-

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Final Remarks

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tions. In particular, it would be of interest to determine whether the elevation in circulating T_4 observed in captivity during vitellogenesis and mating occurs in wild animals. The thyroid hormone content of ridley eggs should also be determined to establish whether maternal hormones appear in yolk. Identification of the location of deiodinases and receptors, useful for establishing thyroid hormone targets and their sensitivity to stimulation, could come from classical studies with tissue salvaged from animals killed for other purposes or from molecular studies of tissue biopsies. Further studies of hypothalamic control of thyroid function, although useful in establishing a linkage between the pituitary-adrenal axis and thyroid, can be achieved only with more invasive in vivo or in vitro techniques. All of this information would provide more effective tools for the evaluation of the impact of human activities on an endocrine gland that is likely involved in the regulation of development and metabolism. Ridley turtles, as fully aquatic species often found near shore, are potentially exposed to a number of anthropogenic chemicals that may impact thyroid function. To evaluate the effects of endocrine-disrupting chemicals on adult, juvenile, or embryonic animals, it is essential that thyroid activity be characterized in populations from relatively pristine habitats as well as in potentially impacted wild populations.

Final Remarks

Despite the challenges of working with ridley turtles, significant progress has been made in the examination of their metabolic and endocrine physiology. Mass nesting behavior and captive rearing programs have provided unique opportunities for investigations of stress, respiratory, and endocrine physiology, which should serve as the foundation for future investigations. Much is left to discover that will help to fill the substantial gaps in our knowledge of the biology of these fascinating reptiles. Of special interest is the arribada phenomenon, unique to the genus *Lepidochelys*. These unexplained large aggregations may have played an evolutionary role in ensuring the survival of the species by conferring individuals with the protection against predators inherent to a large group of conspecifics. However, in modern times these same aggregations could lead to the decimation of the species because animals are particularly vulnerable to anthropogenic activities when on the beach or in coastal waters. The proximity of such large numbers of animals to nesting beaches provides a rare opportunity for an examination of the role of endocrine systems in the regulation of behavior of wild reptiles. Because modern analytical techniques require minuscule amounts of blood for hormone and metabolite measurement, investigators collecting blood samples from wild animals should seek out collaborations that promote the broadest possible analysis of sizable sea turtle blood samples.

It is our hope that the study of the physiology of both species of *Lepidochelys* helps to enhance our understanding of their life histories and to facilitate the design and implementation of improved conservation practices. For example, an improved understanding of the respiratory physiology of the Kemp's ridley may lead to effective protocols to help these animals recover from capture in trawling nets of shrimp boats. Similarly, understanding the role of thyroid hormones in metabolism or thermoregulation may contribute to our ability to assess the impact of exposure to cold waters or to endocrine-disrupting chemicals. In addition, characterization of the endocrine stress response in ridley turtles may help us understand the potential impact of tagging programs, turtle-based tourism programs, incidental fisheries, and egg-harvesting practices on the reproductive biology and behavior of these animals. Importantly, we have no direct data on the relationship between reproduction and stress in ridley turtles. Throughout these studies, it is important to bear in mind the population status of these animals. We must ensure that the need to protect the species is never secondary to the need to understand their physiology.

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