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Basal and Stress-Induced Corticosterone Levels in Olive Ridley Sea Turtles (*Lepidochelys olivacea*) in Relation to Their Mass Nesting Behavior

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ABSTRACT Adrenocortical responsiveness to turning stress was examined in wild, reproductively-active olive ridley sea turtles (*Lepidochelys olivacea*) in relation to their mass nesting (*arribada*) behavior. We hypothesized that the high sensitivity threshold (HST) observed in ovipositing sea turtles is associated with a diminished sensitivity of the hypothalamo-pituitary-adrenal (HPA) axis to stressful stimuli in *arribada* females. We tested this hypothesis by determining whether *arribada* females exhibited an increased activation threshold of the HPA axis to an imposed stressor (turning stress). Mean basal corticosterone (B) and glucose levels were below 1.0 ng/ml and 60 mg/dl, respectively. Basal B remained unchanged throughout a 24-hr period in basking females. Most animals responded to turning stress with elevated mean B levels (up to 6.5 ng/ml after 6 hr) and no increase in circulating glucose. Nearly 50% of females (and none of the males) were refractory to the stimulation. Males exhibited the most rapid response, with B levels significantly elevated by 20 min over basal levels. Among females, *arribada* and solitary nesters exhibited a slower rate of response than basking, non-nesting animals. These results demonstrate that olive ridleys exhibit stress-induced changes in circulating B which are slower than those observed in most reptilian and in mammalian, avian, and piscine species. Furthermore, the presence of refractory females and the relatively slower increase in B in *arribada* and solitary nesters indicate a hyporesponsiveness of the HPA axis to turning stress in nesting olive ridleys. The hyporesponsiveness may be part of a mechanism to facilitate *arribada* nesting. *J. Exp. Zool. 284:652–662, 1999.*

The olive ridley (*Lepidochelys olivacea*) nesting assemblage at Nancite beach, Costa Rica, is composed of highly migratory individuals (Cornelius and Robinson, '86; Plotkin et al., '95). After actively feeding for an undetermined period of time in the eastern tropical Pacific waters, animals with recrudescing gonads migrate to the vicinity of Nancite and group, giving rise to a reproductive aggregation (RA) (Richard and Hughes, '72). This RA occurs during the months of July through December, coinciding with the peak of nesting activity for the Nancite assemblage, when thousands of individuals are seen basking at the surface awaiting an as yet unknown cue to synchronously move onto land about once a month to oviposit (Hughes and Richard, '74; Cornelius et al., '91). Endocrine, satellite telemetry, and tag recovery data indicate that individual olive ridley females typically nest twice within a season (Plotkin et al., '95, '97; S. Cornelius, personal communication, Sonoran Institute).

Mass nesting events (*arribadas*) are unique to the genus *Lepidochelys*. Each *arribada* may include as many as 145,000 individuals and last from 3 to 7 nights (Valverde et al., '98). Given the reduced size of the nesting beach (1 km long) and the large number of participating individuals, the intensity of the phenomenon promotes extensive and frequent physical contact among turtles. Under these circumstances, it might be expected that olive ridleys have developed neurophysiological mechanisms that reduce their sensitivity to disturbance during *arribadas*, allowing them to complete oviposition under chaotic conditions. Preliminary stud-

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ies have shown that crowding during *arribada* events fails to activate the hypothalamo-pituitary-adrenal (HPA) axis of olive ridleys (Schwantes, '86; Valverde et al., '92). Thus, neuroendocrine systems normally activated by stimuli such as crowding stress (i.e., stress system) may be inactive in nesting *arribada* olive ridleys.

The vertebrate stress system functions as a neuroendocrine transducer of environmental information (Stratakis and Chrousos, '95; Denver, '97), playing a major role in the adaptation of the organisms to their changing environment. Environmental perturbations that disrupt organismal homeostasis (stressors) trigger a neurohormonal cascade that results in the elevation of glucocorticoids, catecholamines, and glucose, among other blood factors (Chrousos and Gold, '92), necessary to restore homeostasis. The stress response includes physiological (e.g., inhibition of reproductive and growth functions) and behavioral (e.g., decreased exploratory behavior in novel environments) modifications aimed at adapting to a stressor (Johnson et al., '92; Wingfield, '94). Underlying the stress-induced activation of the HPA axis is basal control activated by endogenous rhythms. Daily cycles in the secretion of glucocorticoids have been observed in most vertebrate species, including reptiles (Dallman et al., '87; Mahapatra et al., '87). If not taken into account, endogenous fluctuations in glucocorticoid secretion may obscure experimental results by confounding the basal activity of the axis with its response to a stressor.

Juvenile sea turtles have been documented to respond to stressors with elevated B levels (Morris and Owens, '82; Aguirre et al., '95; Gregory et al., '96). Nevertheless, published data on adrenocortical activity in response to stress and gluconeogenic capacity of glucocorticoids in reproductively active sea turtles do not exist. This information is of great value to the conservation of these threatened reptiles, as our knowledge of the impact of imposed stressors on endocrine physiology is fragmentary. We therefore undertook a series of experiments to determine whether the responsiveness of the HPA axis to a physical stimulus, turning stress, varied with reproductive behavior in olive ridley sea turtles.

MATERIALS AND METHODS

Study area and animals

The study was conducted at Nancite beach, Santa Rosa National Park, Costa Rica (ca. 10°47′ N; 85°40′ W). The beach is approximately 1 km long and is isolated from human settlements inside a remote valley surrounded by mountains and ocean (see Cornelius et al., '91, for a more detailed description of the area). The animals included in the study were reproductively active adults, unless otherwise stated. Most of the experiments were conducted during July and August prior to the peak nesting months when the animals were gathering to form the RA that is located approximately 1.5 km off the beach. Experimental animals were classified according to the behavior displayed at the time of capture. "Basking turtles" refers to females that were floating at the surface in the RA. "Mating turtles" refers to males that were engaged in mounting or mating behavior in the RA. "Solitary animals" include females that were found nesting at low density when no more than 99 female turtles nested in a single night. "*Arribada* nesters" include those females that participated in a mass nesting event of 100 or more individuals (Cornelius et al., '91). All nesting animals were allowed to fully oviposit before being captured for experimentation. All animals were released into the wild after experimentation was concluded.

Basal corticosterone and glucose levels

Non-nesting animals were sampled to determine the daily range of basal corticosterone (B) levels. Forty basking female olive ridleys were captured in the RA at 0600, 1200, 1800, and 2400 (±0.5 hr) (10 at each time) by means of the turtle rodeo technique (Limpus, '85). This technique consists of leaping off the side of a small boat onto a basking turtle. The animals were then brought on board and a blood sample (20 ml) was drawn immediately from the lateral cervical sinus (Owens and Ruiz, '80). Blood sampling took no more than 6 min, but usually was completed within 4 min of capturing the animals. All diel samples were collected during the months of July and August of 1991. For comparison, a few samples (n = 10) were collected around midnight from nesting females during an *arribada* in August 1990.

To examine the natural fluctuations of B in post-nesting, free-ranging turtles the carapace of 223 nesting females was painted with white quick drying enamel during a single session of the June 1992 *arribada*. Oviposition was confirmed by digging behind the turtle and observing eggs in the nest chamber. All animals were painted between 2300 and 0100 hr during the session. During the subsequent 3 days 11 of these turtles were captured in the RA at different times of the day while basking. Capture and sampling times were recorded to calculate elapsed time from nesting, us-

ing as zero time midnight of the nesting session. A group of 11 turtles sampled during a session of the November 1991 *arribada* were used as nesting reference values.

In an effort to evaluate comparable B levels in gonadally quiescent olive ridleys on their feeding grounds, blood samples were obtained from a group of females that had been captured in the eastern tropical Pacific by means of a dip-net by Robert Pitman (Pitman, '90) during National Marine Fisheries Service (NMFS) research cruises off the coast of Central America. Blood from these turtles was collected within 10 min of capture, centrifuged within 15 min of collection, and frozen immediately in liquid nitrogen; all animals were returned quickly to the ocean. Only a group of 15 females whose testosterone levels were below 20 pg/ml, indicating the presence of quiescent gonads (Plotkin et al., '95), were included in this study.

Turning stress

Twenty nesting female olive ridleys (10 *arribada*, 10 solitary nesters) and 17 basking animals (10 basking females and 7 mating males), were captured at Nancite beach and in the RA and subjected to turning stress. *Arribada* turtles were captured over two different mass nesting events in August and September 1991. Solitary nesting turtles were captured on different dates over a month-and-a-half period (July and August) the same year, whereas basking females were captured within a week in August of 1991. Mating males were captured over a period of a month during June and July 1992. The stress protocol consisted of drawing a blood sample (20 ml) upon capture (samples taken within 1 min from capture with the exception of in-water captures; see Basal levels section) and then turning the animals on their carapaces for 6 hr. Serial blood samples (10 ml each) were drawn at 20, 40, 60, 120, 240, and 360 min from all animals after the initial sample. Samples obtained from animals while on the boat (first three or four samples for basking females and males) were processed between 1 and 2 hr from collection. The remaining samples were obtained on the beach. Blood samples were centrifuged within 15 min for all animals on the beach. Serum was separated and stored frozen in liquid nitrogen until measurement of glucose, corticosterone (B), and testosterone (T).

Corticosterone radioimmunoassay (RIA)

Serum samples were measured using a standard RIA procedure. The B antibody (B3-163, Endocrine Sciences, Calabasas Hill, CA), the radio-labeled (1,2-^{3}H[N]-corticosterone, Dupont/NEN, Wilmington, DE), and the radio-inert (4-pregnen-11β,21-diol-3,20-dione, Steraloids Inc., Wilton, NH) steroids were purchased from commercial suppliers. For the RIA 500 μl aliquots of serum were extracted with 4 ml of ethyl ether. The extraction efficiency was 83 ± 11%. Standard curves were prepared in buffer with known amounts of radio-inert B (1600-0 pg at two-fold dilutions). Final assay incubation volume was 1.1 ml. A pool of serum from male and female olive ridleys captured before and throughout the peak nesting months was included in the B assay for quality control and to calculate intra- and inter-assay variability. The minimal concentration distinguishable from zero (± two standard deviations) was 90 pg/ml. Potency of unknown samples was determined using software (RIAMENU) provided by Dr. Paul Licht (University of California, Berkeley). The B antibody was tested for cross-reactivity by the manufacturing company with desoxycorticosterone (4%), 5β-pregnanedione (1%), progesterone (0.6%), cortisol (0.4%), and less than 0.2% with other 19 steroids tested.

Four different turtle serum samples (three females and one male) plus a buffer and an olive ridley serum pool aliquots (each 500 μl) were supplemented with 8 ng of radio-inert B (40 ng/ml). All samples were serially diluted (250, 125, and 25 μl) and assayed after equalizing all volumes to 500 μl with buffer. All exhibited parallelism to the standard curve. Mean recovery for radio-inert B from these samples was 105.2 ± 3.8 (% ± c.v.). The intra- and inter-assay variabilities of the B assay were 5.1 % and 18 %, respectively.

Testosterone RIA

Testosterone (T) was measured using an RIA similar to the one used for B, with the following modifications. The T antibody (T3-125; Endocrine Sciences), the radio-labeled (1β,2β-^{3}H[N]-testosterone, Dupont/NEN), and the radio-inert (4-androsten-17β-ol-3-one: Steraloids Inc.) steroids were purchased from commercial suppliers. For the RIA, 100 μl samples of serum were extracted with 4 ml of ethyl ether. The extraction efficiency was 93.5 ± 2.3%. Standard curves were prepared in buffer with known amounts of radio-inert T (50-0 pg at two-fold dilutions). Final incubation volume was 1.4 ml. A buffer plus three different pools made from serum of *Chelonia mydas*, *Caretta caretta*, and *L. kempi* captured in different reproductive conditions and different geographic locations, were used for quality control in the assay and to calculate intra- and interassay variability.

The minimal concentration distinguishable from zero was 5 pg/ml. The T antibody was tested for cross-reactivity by the manufacturing company with dihydrotestosterone (44%), Δ-1-testosterone (41%), Δ-1-dihydrotestosterone (18%), 5α-androstan-3β,17β-diol (3%), 4-androsten-3β,17α-diol (2.5%), Δ-4-androstenedione (2%), 5β-androstan-3α,17β-diol (1.5%), estradiol (0.5%), and less than 0.2% with 23 other steroids tested.

Four different turtle serum samples (three females and one male) and an olive ridley serum pool aliquot (each 500 μl) were stripped of any hormone with activated charcoal. Samples were supplemented with 625 pg of radio-inert T (62.5 ng/ml). All samples were serially diluted (100, 25, and 6.25 μl) and assayed after equalizing all volumes to 100 μl with buffer. All exhibited parallelism to the standard curve. Mean recovery for radio-inert T from these samples was 105.0 ± 14.0 (% ± c.v.). The intra- and inter-assay variabilities of the T assay were 6.5% and 7.4%, respectively.

Glucose assay

Serum glucose was measured by means of a commercial colorimetric assay (Sigma; colorimetric glucose kit using o-Toluidine, catalog no. 635). A glucose standard curve was prepared by serially diluting a stock solution of glucose (100 mg/dl) to 100, 75, 50, 25, and 5 mg/dl. A 25-μl aliquot of serum diluted to 50 μl with distilled water or 50 μl of standard and 2.5 ml of o-Toluidine reagent were mixed and then boiled (100°C) in a water bath for 10 min. The absorbance of the samples and standards was measured (λ = 635 nm) after cooling for 10 min in a water bath at room temperature. A linear regression was performed with the program BIOASSAY (provided by Dr. Paul Licht, University of California at Berkeley) to determine the concentration of glucose in the serum samples. The intra- and inter-assay variability were 6.6% and 14.0%, respectively.

Statistical analysis

All statistical analyses were performed under SAS/PC statistical package, version 6.08, SAS Institute, Inc., Cary, NC. All analyses were performed at a level of significance (α) of 0.05. Data sets including animals that were sampled on only one occasion were analyzed by means of an analysis of variance (ANOVA) and a posteriori Tukey tests. Data sets where the same animals were serially bled were log-transformed and analyzed by a repeated measures ANOVA (rmANOVA). To determine the time at which mean values were different from those measured at zero time, a CONTRAST transformation using mean zero time values as controls was applied. If the initial rmANOVA rejected the null hypothesis of within subject effects of no interaction between TIME*TURTLE CATEGORY, then the univariate arrangement of the data was converted to a multivariate, log-transformed form. This arrangement of the data was followed by a Tukey's studentized range test comparing mean levels of the different turtle categories at each specific time. In addition, a Dunnett one-tailed *t*-test was performed on these data sets comparing the mean values measured at zero time (basal) with those measured at the subsequent times, within each of the turtle categories.

RESULTS

Basal B levels

Diel basal B levels in basking female olive ridley sea turtles did not vary significantly over time, with a mean value of 114.2 ± 10.2 pg/ml (Fig. 1). Mean B levels in *arribada* nesting animals were significantly elevated ($P = 0.0012$) with respect to basking animals sampled at midnight.

B levels for a group of post-nesting females (white painted) along with B levels from a reference group sampled immediately after nesting during a single night of the June 1992 *arribada* are shown in Fig. 2. No significant differences were detected in B levels among females captured at up to 80 hr after nesting, although small sample

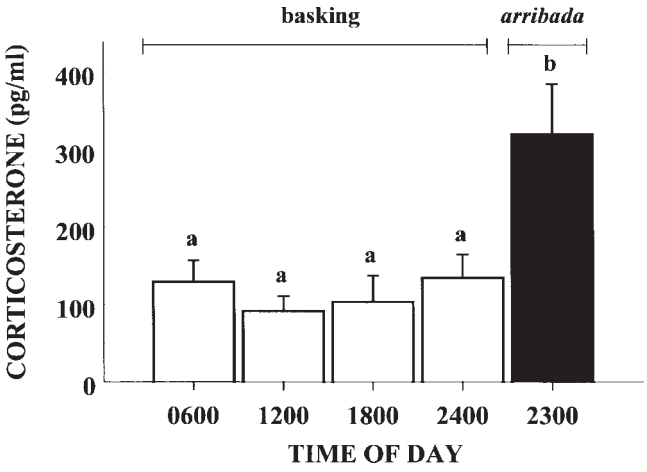

Fig. 1. Mean basal corticosterone levels (±SEM) in basking female olive ridley sea turtles captured at different times of the day (n = 10 for each bar). Solid black bar corresponds to females sampled during an *arribada* immediately after oviposition. Different letters above means indicate statistically significant differences among values ($P = 0.0012$).

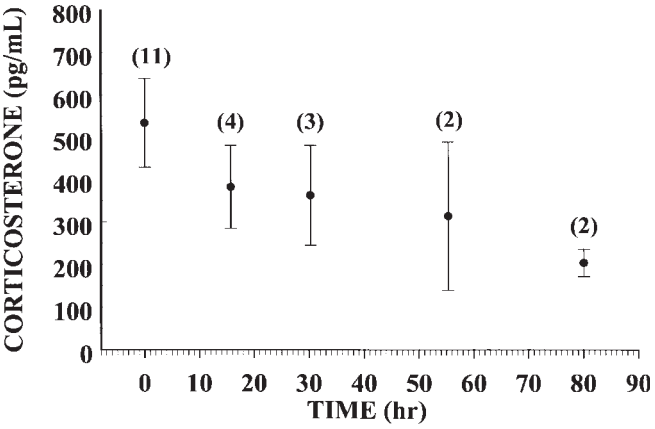

Fig. 2. Mean B levels in nesting and post-nesting free-ranging female olive ridleys captured at Nancite beach and adjacent waters. Parenthetical numbers indicate sample sizes. Means are not significantly different ($\alpha = 0.05$).

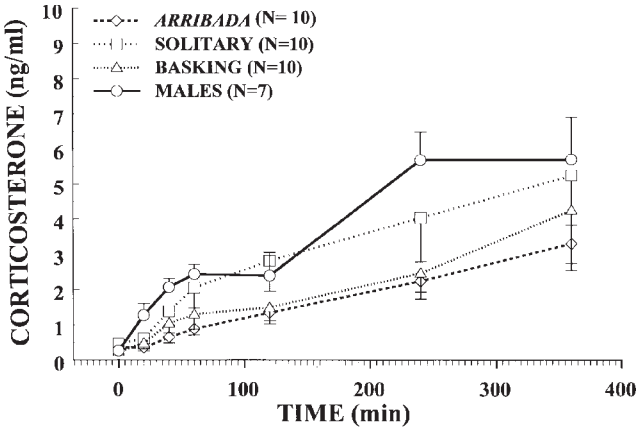

Fig. 3. Mean circulating B levels in male and female olive ridleys in response to turning stress. Groups were classified according to the behavior displayed at the time of capture. Parenthetical values indicate sample sizes. No statistically significant differences were found among the different groups ($\alpha = 0.05$).

sizes at later times may have precluded detection of significant differences.

Turning stress

Mean B levels measured in *arribada* and solitary nesters, and in basking females did not increase over the first 20 min in response to turning stress. In contrast, mating males exhibited a significant increase in B by 20 min (Table 1). In all treatment groups mean B levels remained below 6 ng/ml during the experiments (Fig. 3). Statistical analysis indicated that turtle groups had reached similar endpoints by the term of the experiments. However, the analysis demonstrated a significant interaction between time and turtle group ($P = 0.0022$), as well as a significant effect of time ($P = 0.0001$). These observations indicate that the rates of B increase in the four groups were different and that B rose significantly over time in all groups with respect to basal levels.

TABLE 1. Serum mean B levels at times zero (basal) and 20 min and their ratio in olive ridleys subjected to turning stress[1]

		B levels (pg/ml)		
	Sample size	T_0	T_{20}	Ratio T_{20}/T_0
Arribada	10	328	322	0.98
Solitary nesters	10	463	603	1.30
Basking females	10	233	476	2.04
Males	7	266	1,269*	4.77

[1]For each group a Dunnett one tailed *t*-test was applied to determine whether B levels were elevated at 20 min with respect to basal levels. Significant differences at $P = 0.0001$ are designated by an asterisk.

Mean B levels were significantly elevated in *arribada* nesters by 120 min ($P = 0.0001$), by 60 min in solitary nesters ($P = 0.0001$), by 40 min in basking animals ($P = 0.0001$), and by 20 min in mating males ($P = 0.0001$), with respect to basal levels of each group.

The variability observed in the mean B values for *arribada* and solitary nesters, and for basking females was in part due to the presence of animals that exhibited a diminished response to stimulation, i.e., maximal absolute B levels did not rise above 1.6 ng/ml by the end of the experiment. All males responded with B levels higher than 1.6 ng/ml. Accordingly, animals within each female group were divided a posteriori into two subgroups, designated as responsive (above 1.6 ng/ml) and unresponsive (below 1.6 ng/ml) (Fig. 4). Analysis by subgroups indicated that *arribada* and solitary nesters and basking females responsive and unresponsive animals exhibited statistically significant differences ($P = 0.0015$, $P = 0.0005$, and $P = 0.0335$, respectively). B levels increased significantly in responsive animals in relation to unresponsive females as no significant interaction between time and subgroup was detected.

Serum T was measured in samples obtained from every group at time zero as an index of ovarian status to test for the effect of the ovary in modulating adrenal responsiveness (Fig. 5). Mean T levels for responsive and unresponsive animals of all categories exhibited no statistically significant differences.

Circulating glucose levels were measured in the

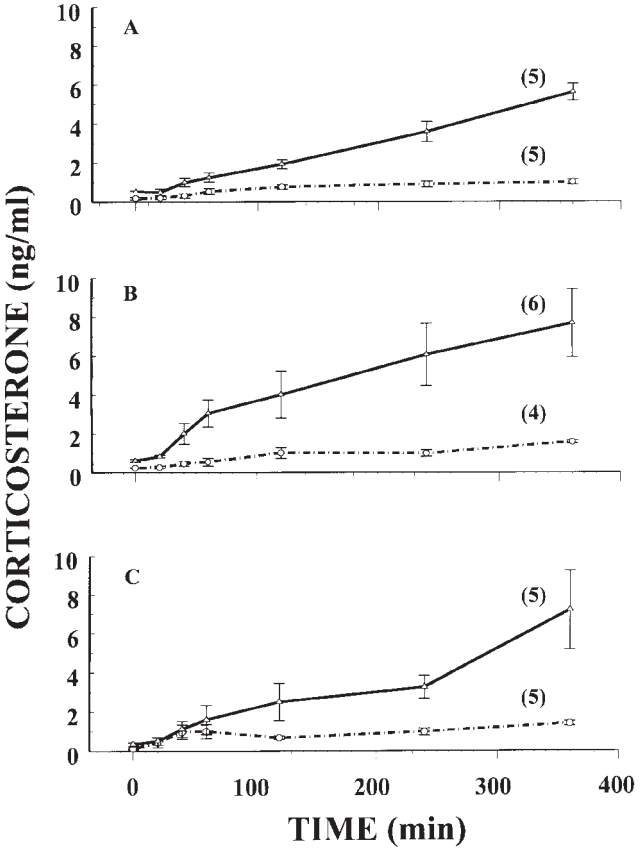

Fig. 4. Mean circulating B levels in *arribada* (**A**) and solitary (**B**) nesters and basking females (**C**) subjected to turning stress. Data from Fig. 3 were reclassified into responsive (solid line) and unresponsive (hatched line) turtles and redrawn. Subgroups were consistently different over time (P = 0.0015, P = 0.0015, and P = 0.0335, respectively). Parenthetical numbers indicate sample sizes.

four groups of turtles subjected to turning stress and in the group of 15 gonadally quiescent (mean T levels = 11.9 ± 10.4 pg/ml) female olive ridleys captured in the eastern tropical Pacific during NMFS cruises (Fig. 6). Glucose levels for these olive ridleys were significantly higher than those of all Nancite turtles examined. The following is a description of the analysis for the Nancite animals subjected to turning stress, exclusively. Mean glucose levels in all groups of turtles subjected to turning stress were statistically indistinguishable at the end of the experiments. However, the statistical analysis indicated a significant interaction between time and turtle group (P = 0.0003), as well as a significant effect of time (P = 0.0167). These indicate that glucose levels varied differently in the four groups of turtles and that the initial values were different from those measured in the subsequent

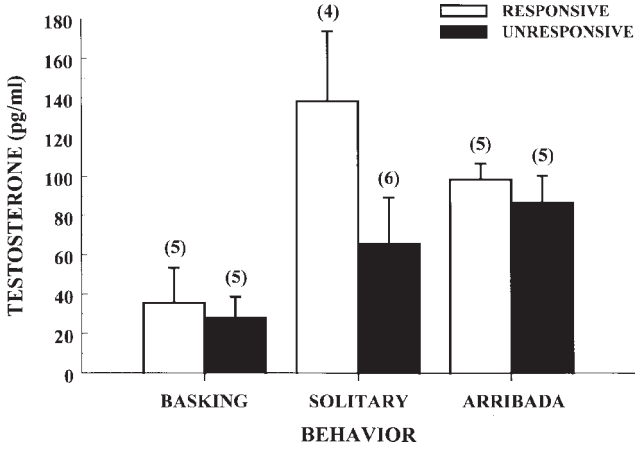

Fig. 5. Mean circulating T levels in responsive and unresponsive female olive ridley sea turtles as a function of their reproductive behavior. T was measured in samples collected before subjecting the animals to turning stress. Parenthetical numbers indicate sample sizes. No statistically significant differences were found between responsive and unresponsive animals in T levels within each behavioral category (α = 0.05).

samples. Further statistical analysis revealed that mean glucose levels in basking females were significantly lower with respect to those of *arribada* nesters measured at time zero (P = 0.0043). In addition, there was a statistically significant decrease in mean glucose levels in solitary nesters by 360 min with respect to initial levels (P = 0.0059).

DISCUSSION

All sea turtle species have in common a high sensitivity threshold (HST) period during nesting

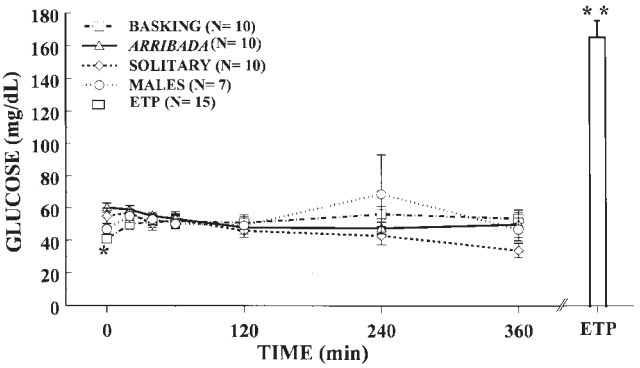

Fig. 6. Mean circulating glucose levels in mating male and basking female and in *arribada* and solitary nesting olive ridley sea turtles subjected to turning stress. On the abscissa ETP stands for eastern tropical Pacific. Mean glucose in these gonadally quiescent females represent basal levels. Single asterisk indicates significant statistical differences between glucose levels of basking and *arribada* females at time zero (P = 0.0043). Two asterisks indicate that ETP values were significantly higher than those of males at 240 min (P = 0.005).

when turtles are behaviorally refractory to physical stimuli such as touch, light, and sound (Carr and Hirth, '62; Ehrenfeld, '79). Although the climax of this insensitivity is reached during the act of oviposition (Hendrickson, '58; Hirth, '71), this period appears to have been extended uniquely in *arribada* olive ridleys to encompass the time when females are searching for a nesting site. Indeed, *arribada* olive ridleys have been described as less sensitive than conspecifics nesting solitarily (Hughes and Richard, '74). The HST period may play a significant role in allowing turtles to nest during the chaotic *arribadas*. In the present study we tested the hypothesis that during nesting *arribada* turtles possess a hyporesponsive HPA axis in relation to other turtles. Thus, we developed a "turning stress" protocol, analogous to the "capture stress" protocol used in birds (Wingfield et al., '98), to evaluate the relative activity of the HPA axis of olive ridleys under different reproductive behavior paradigms. Our results showed that within 2 hr from the onset of turning stress nesting turtles responded more slowly with increased mean B levels in relation to non-nesting turtles. Interestingly, glucose levels did not increase concomitantly with elevated B levels in turned turtles. In addition, we failed to observe a nychthemeral rhythm in basal B levels in gravid turtles basking in the water.

Elevated adrenocortical secretion of B has been extensively used in birds as an index to compare inter- and intra-specific adrenal responsiveness to capture stress in relation to basal B levels (Wingfield, '94). Using this methodology, a suite of factors, including body condition and social status, have been observed to play an influential role in the rate of increase of B in birds (Wingfield et al., '98) and lizards (Dunlap and Wingfield, '95), although no single factor appears to be a universal regulator of the rapidity of the response. The rate of adrenocortical response is of particular importance to vertebrate organisms because glucocorticoids are thought to mediate the compensatory physiological mechanisms responsible for restoring homeostasis (Johnson et al., '92). In the present study, consistent application of the turning stress protocol allowed us to establish significant differences in the rate of increase of B with respect to basal levels among four different behavioral categories. Our results indicate that the HPA axis of olive ridleys is responsive to turning stress, as most individuals, including all the males, exhibited increased B over basal levels after the onset of the experiments. B levels remained low relative to nesting levels in post-nesting, free-ranging animals hours after returning into the ocean (white painted females). These data are consistent with the idea that B levels increase in response to turning stress and not spontaneously in post-nesting animals. Data showed that *arribada* and solitary nesters exhibited elevated B over basal levels by 120 and 60 min, respectively, from capture and turning, whereas basking females responded by 40 min. These results are consistent with the hypothesis of a diminished responsiveness of the HPA axis to stress in mass nesting turtles. This hyporesponsiveness may be a consequence of the protracted HST period and may contribute to the effective nesting of the turtles under crowded conditions. Because the nesting olive ridley's adrenal gland is readily responsive to adrenocorticotropic hormone injections (Valverde, '96), this diminished responsiveness may be expressed at a central or pituitary level.

The adrenocortical response of sea turtles appears to be substantially slower than that of other vertebrates. Indeed, adrenocortical activation appears to be particularly dramatic in stressed birds and fish, even at a young age, increasing several times over basal levels within a few minutes from subjecting the animals to capture and handling stress (Barry et al., '95; Kakizawa et al., '95). In birds, a significant increase of B over basal levels in response to handling may occur as fast as 45 sec from the onset of the stimulus, but in general appears to occur within 15 min, varying according to the species (as reviewed by Le Maho et al., '92; Wingfield et al., '95). In contrast few studies are available in reptiles. In the tree lizard (*Urosaurus ornatus*), B increased nearly seven times over basal levels by 10 min in response to capture (Moore et al., '91). Western fence lizards (*Sceloporus occidentalis*) have been reported to exhibit a significant elevation in mean B over basal values after 10 min of capture (Dunlap and Wingfield, '95). Adrenocortical responsiveness to stressors has been examined in loggerhead sea turtles (Morris and Owens, '82; Gregory et al., '96) and in the freshwater slider turtle (*Trachemys scripta*) (Cash et al., '97). Unfortunately, the rapidity of the response in relation to basal B levels could not be determined in these studies. Together, these data indicate that gravid female olive ridleys possess a less sensitive HPA axis given that basking and nesting animals required between 40 min to 2 hr to exhibit elevated mean B levels. Interestingly, in the present study male olive ridleys exhibited a significant increase of B by 20

min, indicating that males can respond more rapidly to turning stress than female olive ridleys. These data further support the hypothesis of adrenocortical hyporesponsiveness in nesting olive ridleys and suggest a modulatory ovarian influence on the cheloniid HPA axis.

In the present study, nearly 50% of female olive ridleys did not exhibit a significant elevation in circulating B with respect to basal levels in response to turning stress. Other unresponsive females were also found in subsequent years in lower proportion with respect to responsive animals (Valverde, '96). This finding may be exceptional among vertebrates. It is possible that the refractoriness to turning stress of individual females is regulated by gonadal influences. Gonadal modulation of adrenal output has been shown in many vertebrates, including reptiles and birds (Greenberg and Wingfield, '87; Wingfield et al., '95). Unfortunately, the specific mechanisms underlying these modulatory effects remain poorly understood in nonmammalian vertebrates. We attempted to examine the effect of the ovary on the sensitivity of the HPA axis in our experimental animals. To classify the ovarian condition of our animals, T levels were measured in samples obtained at time zero from all female turtles. T appears to be a good indicator of ovarian status in ridley sea turtles as maximal levels gradually decrease as the ovary decreases in size with each ovulation (Rostal et al., '97; Rostal et al., '98). Thus, in our study population high T levels (>20 pg/ml) corresponded to females at the beginning of their nesting season, whereas animals with low T levels (i.e., <20 pg/ml) had completed their last nest of the year (Plotkin et al., '95, '97). Analysis of T titers indicated that adrenocortical refractoriness of the individuals included in the study was not related to ovarian condition since both refractory and responsive females possessed similar T values. Nevertheless, since not a single male was found to be unresponsive to the treatment, the influence of ovarian hormones cannot be completely disregarded.

An alternative explanation for the presence of females refractory to turning stress in the present study is the age of the animals. It has been shown that non-senescent older rats exhibit a diminished responsiveness of central and peripheral catecholaminergic systems to acute stress, concomitantly with progressive hypothalamic corticotropin-releasing hormone (CRH) deficiency (Cizza et al., '95). This observation is consistent with data from smaller sea turtles which are capable of responding faster to the stress of acute captivity than larger turtles, if smaller individuals corresponded with younger animals and larger turtles with older animals in the study (Gregory et al., '96). It is thus interesting to speculate that the unresponsive turtles constitute a group of older individuals whose hypothalamic responsiveness to stressors is impaired due to a central CRH deficiency. Unfortunately, no techniques are currently available for determining the age of wild turtles (Zug et al., '97).

A classical component of the stress response of vertebrate species is the elevation in circulating glucose levels as a consequence of the stimulation of hyperglycemic hormones, such as catecholamines and glucocorticoids (Devenport et al., '89; Mizock, '95). The hyperglycemic effect of glucocorticoids under stressful conditions in many vertebrate species is due to their well-documented gluconeogenic capacity and their ability to inhibit peripheral glucose utilization as well as the conversion of liver glycogen into glucose (Widmaier, '90; Mizock, '95). In reptiles, B has been demonstrated to possess gluconeogenic and hyperglycemic effects (Callard and Chan, '72; Jacob and Oomen, '92). Our results indicate that circulating glucose levels did not increase in the olive ridleys subjected to turning stress in spite of elevated mean B levels. Similar results have been found in mammalian, reptile, and fish species, in which blood glucose appears to be dissociated from glucocorticoid levels (Yamada et al., '93; Carragher and Rees, '94; Dunlap and Wingfield, '95), although the mechanisms underlying this dissociation are not understood. Results indicate that B may not be a hyperglycemic hormone in gravid or gonadally depleted olive ridley sea turtles. The lack of response may in part be related to the hypophagic behavior observed in these turtles during the reproductive season (Owens, '76, '80), a time when gluconeogenic substrates may be limited due to the decreased food intake and the effort invested during the migration to the nesting beach. Support for the hypothesis of hypophagia comes from the observation that basal and maximal glucose levels exhibited by turtles subjected to turning stress are at least 2.4-fold lower than those of presumptive gonadally quiescent females captured at the feeding grounds in the eastern tropical Pacific. In addition, glucose levels of stressed individuals were approximately half of basal levels measured in juvenile green sea turtles (*Chelonia mydas*) and juvenile and adult loggerhead sea turtles (*Caretta caretta*) (Bolten and Bjorndal, '92; Bolten et al., '92; Aguirre et al., '95)

captured at their feeding grounds. It is important to note that some glycolysis may have occurred in the samples collected from males and basking females while samples were en route to centrifugation at our base camp on the beach. Glycolysis is thought to occur at a rate of about 5% at room temperature in the presence of red blood cells (Meites and Bohman, '63). Glycolysis may account for the differences observed at time zero between basking females and *arribada* nesters. However, we feel it is unlikely that glycolysis can account for the observed lack of hyperglycemia due to the rapidity with which we processed the samples on land.

Glucocorticoid diel rhythms are pervasive among vertebrates and have been reported in fish (Peter et al., '78), amphibians (Thurmond et al., '86), reptiles (Lance and Lauren, '84), birds (Davis and Siopes, '89), and mammals (Walker, '94), although an exception among reptiles has been reported (Tyrrell and Cree, '88). In the present study, we failed to identify a diel rhythm in B levels. The possibility exists that we missed a peak during our 6-hr interval sampling. However, we feel that this is not the case since less than 1% of the hundreds of turtles that our team sampled at the nesting site over the years exhibited basal B levels higher than 1 ng/ml. In contrast, diel cycles in the basal secretion of B have been described in 5 month old loggerhead (*Caretta caretta*) sea turtles, with a single peak in mean B levels of approximately 3.0 ng/ml at 0530 hr in animals born and raised in captivity and fed on a daily basis (Schwantes, '86). It is possible that the lack of diel B cycles observed in the present study is a further consequence of the hypophagic behavior of gravid olive ridleys, as only organisms with well-defined daily activity and feeding regimens are believed to exhibit daily variations in the secretion of B (Thurmond et al., '86). Interestingly, it has been shown that diel melatonin rhythms are absent in gravid female green turtles, but are present in juvenile and reproductively active male greens (Owens et al., '80). These data suggest the hypoactivity of central rhythms in gravid cheloniids. This hypoactivity might be linked to the lack of a diel rhythmicity of B and may partially explain the hypofunction of the HPA axis reported in the present study.

In conclusion, reproductively active olive ridley sea turtles located in the vicinity of the nesting beach exhibit a relatively insensitive HPA axis to turning stress. This insensitivity is strongest during the perinesting period and may have evolved to facilitate nesting during the chaotic *arribadas*. This mechanism may be unique to ridley turtles or may be pervasive among nesting sea turtles.

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