Vol. 53: 533–545, 2024
https://doi.org/10.3354/esr01323

ENDANGERED SPECIES RESEARCH
Endang Species Res

Published April 25

Endocrine data provide further evidence of physiologic derangement in sea turtles affected by the *Deepwater Horizon* oil spill

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ABSTRACT: This study was conducted to characterize the adrenocortical and thyroid status of Kemp's ridley sea turtles *Lepidochelys kempii* that were affected by the *Deepwater Horizon* oil spill. A plasma aldosterone assay was validated for *L. kempii*, and it was used along with previously validated assays for corticosterone and free thyroxine to assess hormone concentrations of 30 *L. kempii* that were hospitalized due to oil exposure, representing 2 severities of oiling (lightly or heavily oiled). Hormone concentrations were also assessed in relation to 8 clinical biochemical analytes. Analysis of paired samples indicated that oiled turtles had significantly higher initial aldosterone and corticosterone concentrations, which declined during convalescence (average 96 and 90% decrease, respectively). Thyroxine concentrations significantly increased between admission and convalescence (average 65% increase). Initial biochemical data indicated significantly higher plasma potassium, ionized magnesium, and lactate concentrations compared to convalescent values. Aldosterone concentrations were positively correlated with corticosterone, negatively correlated with free thyroxine, and variably correlated with several clinical biochemical analytes. Results of this study indicate that *L. kempii* had robust adrenocortical activity after oiling, capture, and transport to the hospital, regardless of the degree of oiling, resulting in very high plasma concentrations of aldosterone and corticosterone. This study also confirms that aldosterone can be reliably measured in sea turtle plasma samples, providing another diagnostic tool for the physiologic assessment of this Critically Endangered species.

KEY WORDS: Oil spill · Sea turtle · Adrenal · Aldosterone · Corticosterone · Thyroid

1. INTRODUCTION

Sea turtles are globally threatened (Wallace et al. 2011, IUCN 2023), having been negatively impacted by fisheries interactions (Wallace et al. 2013), habitat loss (Wallace et al. 2011), non-sustainable hunting and consumption of eggs (Wallace et al. 2011), vessel collision (Foley et al. 2019), weather events (Roberts et al. 2014, Griffin et al. 2019), pollution (Stacy et al. 2017, Wallace et al. 2017), and disease (Aguirre et al. 1995). The smallest of the 7 species, Kemp's ridley turtle *Lepidochelys kempii*, is Critically Endangered (IUCN 2023), having experienced a 99% decline in its nesting population between the 1940s and 1980s (Bevan et al. 2016). Adults of this species are primarily found in the Gulf of Mexico, nesting on beaches in

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Mexico and to a lesser extent in Texas, USA. Conservation efforts in the USA and Mexico were successful in recovering the species from near extinction, but the nesting population remains well below historical estimates, with fluctuating nesting trends in recent years (Bevan et al. 2016).

Injured and ill sea turtles are often presented to rehabilitation facilities for veterinary evaluation and care, with hope of recuperation and return to the wild. Assessment of the physiologic status of sea turtles by blood analysis is commonplace and provides important prognostic information (Innis et al. 2009, Keller et al. 2012, Stacy et al. 2013). Corticosterone is the primary glucocorticoid hormone produced by the chelonian adrenal gland in response to stressors, and elevated concentrations of corticosterone have been described for sea turtles affected by various disease states, fisheries interactions, injuries, capture, and long-distance transportation (Gregory et al. 1996, Jessop et al. 2000, 2002, Jessop & Hamann 2004, Ray et al. 2008, Hunt et al. 2012, 2016a,b, 2019, 2020, Flower et al. 2015). Concentrations of several thyroid hormones have been evaluated in sea turtles, including total thyroxine (T4; Licht et al. 1985, Moon 1992, Moon et al. 1998, 1999, Rostal et al. 1998, Valente et al. 2011), triiodothyronine (T3; Moon 1992, Moon et al. 1998, 1999), and free thyroxine (fT4; Hunt et al. 2012, 2016a,b, 2019, 2020), and concentrations may be suppressed or elevated during certain disease states (Hunt et al. 2012, 2016a).

The adrenal mineralocorticoid, aldosterone, is found in all reptilian orders and promotes urinary sodium retention and potassium excretion, as typical of other vertebrates (Macchi & Phillips 1966, Mehdi & Carballeira 1972, Bradshaw & Grenot 1976, Duggan & Lofts 1978, Brewer & Ensor 1980, Uva et al. 1982, Balment & Loveridge 1989). Although a seemingly important subject of study for reptiles that live in a marine environment, there is only a single report of plasma aldosterone concentrations for 4 individual *L. kempii* (Ortiz et al. 2000). Studies of several marine mammal species indicate that aldosterone concentrations correlate positively with glucocorticoid concentrations and increase in response to stressors (Burgess et al. 2017, Champagne et al. 2018, Bergfelt et al. 2020).

The 2010 *Deepwater Horizon* (DWH) oil spill in the Gulf of Mexico is estimated to have exposed 100 000's of sea turtles to oil and to have killed up to 86500 juvenile *L. kempii* (DWH NRDA Trustees 2015, McDonald et al. 2017, Wallace et al. 2017). During the DWH spill, 319 live oiled sea turtles, including 192 *L. kempii*, were recovered at sea and transported to rehabilitation facilities for medical assessment and management. Blood biochemical analysis revealed nonspecific metabolic and osmoregulatory disturbances that were likely due to exertion, physical exhaustion, hyperthermia, and dehydration, secondary to the cumulative effects of oiling, capture, and transport (Stacy 2015, Stacy & Innis 2015, Stacy et al. 2017). In addition to these documented derangements, retrospective toxicologic considerations suggested that oiled turtles may have also experienced impaired adrenal gland function, based on data for other species evaluated during the DWH oil spill, as well as laboratory observations of DWH oil exposure in surrogate (nonmarine) chelonian species (Mitchelmore et al. 2017, Takeshita et al. 2021).

Given the productive use of endocrine data for the assessment of marine turtles exposed to other stressors, as well as speculation about adrenal impairment, we utilized archived, frozen plasma samples to assess the adrenal and thyroid status of *L. kempii* that were evaluated during the DWH oil spill. We hypothesized that plasma concentrations of corticosterone, aldosterone, and fT4 would be elevated upon admission to the hospital, that concentrations of these hormones would be greater in more heavily oiled turtles, and that concentrations of these hormones would normalize (decrease) during convalescence. We also hypothesized that concentrations of these hormones would correlate with clinical biochemical data.

2. MATERIALS AND METHODS

2.1. Study animals and blood sample collection

Turtles included in this study were recovered at sea in the northern Gulf of Mexico during federal response operations and transported by vehicle to Audubon Nature Institute, New Orleans, LA, USA, for rehabilitation between 31 May and 1 August 2010 (Stacy 2015). Transportation time in vehicles was approximately 1.5 h (Stacy et al. 2017). Upon arrival at the rehabilitation center, turtles were examined, and an initial blood sample was collected from the external jugular vein for point-of-care biochemical analysis prior to bathing and therapeutic intervention. After point-of-care analysis, remaining blood was transferred into lithium-heparin tubes and centrifuged (10 min at 5000 × *g*) to harvest plasma. Samples were initially frozen at −20°C for approximately 5 mo and then archived at −80°C until analysis. Blood samples were also collected (external jugular vein) and analyzed at various time points during rehabilitation for hematologic and plasma biochemical analysis as

directed by the attending veterinarians (Stacy et al. 2017). The elapsed time between sample collection relative to initial handling was not recorded.

For the present study, we selected archived plasma samples from a subset of hospitalized, juvenile Kemp's ridley turtles for which paired initial (acquired on Day 0) and convalescent samples (acquired after 30–59 d of hospitalization) were available, including 15 turtles that were categorized as 'lightly oiled' (a thin layer of oil lightly covered multiple parts of the body, and thicker aggregated oil, if present, was focally distributed) and 15 turtles that were categorized as 'heavily oiled' (aggregates of thick, tenacious oil diffusely covered the body) (Stacy 2015). Turtles ranged in body weight from 0.86 to 3.85 kg (mean 1.36 kg, median 1.32 kg), and had straight carapace length of 17.5 to 35 cm (mean 20.9 cm, median 20.8 cm). All turtles included in the present study survived to be released to the wild. Due to the extremely low mortality rate of turtles that were hospitalized during the DWH oil spill (4 deaths out of 319 hospitalized turtles), and extenuating circumstances of those few mortalities, the present study did not evaluate turtles that died during hospitalization (Stacy et al. 2017).

2.2. Hormone analysis

Frozen plasma samples were shipped on dry ice overnight to the endocrine laboratory at the New England Aquarium, Boston, MA, and stored at −80°C until analysis. Commercial ^{125}I radioimmunoassay kits (RIA; MP Biomedicals) that had been previously validated for *Lepidochelys kempii* were used for the quantification of corticosterone (corticosterone double antibody RIA kit, catalog #07-120103) and fT4 (fT4 coated tube RIA kit, catalog #06B-257214) (Hunt et al. 2012). Standards (corticosterone: 0.0625–5 ng ml^{-1}, 7 standards; fT4: 1.5–120 pg ml^{-1}, 6 standards) and samples (corticosterone: plasma diluted at 1:10 with steroid diluent; fT4: plasma undiluted, 1:1 neat) were assayed according to manufacturer instructions with minor modifications by Hunt et al. (2012), including performing the corticosterone assay at 50% volume (i.e. all samples, standards, and detection reagents were used at half the volume outlined in the kit protocol), as well as inclusion of an additional low-concentration standard. Each tube was counted for 2 min in a gamma counter (Wallac 1470 Wizard®; PerkinElmer Life Sciences) for quantification, with the final result adjusted for dilution factor, if necessary.

Aldosterone was quantified in extracted plasma using a commercial enzyme immunoassay kit (DetectX® aldosterone kit, catalog #K052, Arbor Assays). In preparation for aldosterone measurement, plasma was extracted following the manufacturer-recommended protocol. In brief, 250 µl of ethyl acetate were added to 250 µl of plasma in a glass tube. Samples were vortexed for 30 s until homogenized and then let stand to allow layers to separate. The top organic layer was carefully aspirated and placed into a clean glass tube. The extraction steps using ethyl acetate were repeated 2 more times, pooling the supernatant layers. The final resulting ethyl acetate supernatant was evaporated to dryness under compressed air using a Reacti-Vap™ Evaporator (Thermo Fisher Scientific) and then reconstituted in 250 µl of assay buffer (catalog #X065, Arbor Assays) prior to hormone analysis. This enzyme immunoassay was analytically validated for *L. kempii* plasma samples by testing for: (1) parallelism between serial dilutions of pooled plasma extract (1:1 to 1:32) and the standard reference curve; and (2) accuracy (i.e. matrix effect) in results of standards spiked with pooled plasma extract vs. unspiked standards. Assay method was performed according to manufacturer's instructions using the overnight protocol (i.e. incubating overnight at 4°C), except that an additional low-concentration standard was included in the standard curve to increase the detection range (aldosterone: 0.98–4000 pg ml^{-1}, 7 standards). Optical density was read at 450 nm using a microplate spectrophotometer (Epoch with Gen5™ software; BioTek Instruments).

All samples, standards, and controls were assayed in duplicate. Any sample with a coefficient of variation between duplicates >10% was re-assayed. Quality control in assays (i.e. precision and reproducibility) was monitored by measuring the concentration of high (~30% binding) and low (~70% binding) control samples in each assay. Inter-assay coefficients of variation were aldosterone: 8 and 10% (n = 9 assays); corticosterone: 7 and 8% (n = 5 assays); and fT4: 3 and 4% (n = 4 assays) for high and low controls, respectively. As reported by the manufacturer, the corticosterone antibody had 100% cross-reactivity with corticosterone, 0.34% with desoxycorticosterone, 0.10% with testosterone, and ≤0.05% with 18 other steroids, including cortisol and aldosterone; the thyroxine antibody had 100% cross-reactivity with L-thyroxine, 91% with D-thyroxine, 3% with 3,3′,5-triiodo-L-thyronine (T_3), 8% with 3,3′,5′-triiodo-L-thyronine (rT_3), 0.05% with 3,5-diiodo-L-thyronine (T_2), and <0.01% with 6 other tested compounds; the aldosterone antibody had 100% cross-reactivity with aldosterone, 0.05% with corticosterone, 0.02% with desoxycorticosterone, and <0.02% with 5 other steroids including cortisol.

Final plasma concentrations for corticosterone are reported as ng ml^{-1} of immunoreactive hormone, whereas fT4 and aldosterone data are reported as pg ml^{-1} of immunoreactive hormone. Plasma hormone assays for aldosterone, corticosterone, and fT4 were completed for all samples, except for 1 sample that had insufficient remaining volume for corticosterone measurement and 4 samples with insufficient volume for fT4 measurement (see Table S1 in the Supplement at www.int-res.com/articles/suppl/n053p533_supp.pdf). Corticosterone concentrations were measurable in all available samples. Three convalescent samples had non-detectable aldosterone and 2 convalescent samples had non-detectable fT4, indicating that concentrations in these samples were below the limit of assay detection (Table S1). For statistical analysis, concentrations for these samples were assigned a nominal value that was half of the quantification limit (i.e. aldosterone value of 2.49 pg ml^{-1} and fT4 value of 0.23 pg ml^{-1}) to avoid non-random missing data (Beal 2001).

2.3. Biochemical analysis

After acquiring hormone data, if remaining plasma volume allowed, biochemical data were generated using the Stat Profile Prime Plus VET Critical Care Analyzer (NOVA Biomedical; Table S1), including sodium, potassium, chloride, ionized calcium (iCa), ionized magnesium (iMg), glucose, lactate, blood urea nitrogen (BUN), and calculated sodium:potassium ratio (n = 24 initial samples, n = 17 convalescent samples, representing 28 turtles; Table S1). Neither initial nor convalescent plasma samples were of sufficient volume for biochemical analysis for 2 turtles (Table S1).

2.4. Statistical analysis

Immunoassay validation was assessed for parallelism by plotting percentage of antibody bound (%Bo/B0) against log(relative dose) and using an *F*-test to compare the slopes of the linear portions of both binding curves (i.e. assay standards and serially diluted extract); accuracy was assessed using linear regression to evaluate slope and linearity of observed vs. expected dose. Descriptive statistics (mean ± SD) were used to summarize the data set. Aldosterone and corticosterone concentrations were transformed using the common logarithm, log_{10}, to adjust for a skewed, non-normal distribution. Levene tests were conducted for all variables to check for homogeneity of variance, using Welch's correction for unequal variances. To examine the effect of oil exposure on the physiologic state of turtles, we compared initial hormone concentrations (aldosterone, corticosterone, or fT4) across oiling categories using univariate ANOVA. Repeated measures ANOVA models were used to evaluate temporal changes in hormone concentrations over time for individual turtles (initial vs. convalescent values), and the potential influence of the oiling category. We performed Spearman's rank-order correlations to evaluate the relationship between plasma hormone and biochemical analytes. In order to ensure that observations included in this analysis were independent, we used unpaired data comprised of either initial or convalescent data for each turtle. Determining this data set required the exclusion of 2 initial sample results, which were randomly selected to ensure that individual turtles were represented only once in this analysis. Differences were considered significant at $p < 0.05$. Post hoc Bonferroni tests of pairwise multiple comparisons were used to identify significant differences. All statistical analyses were performed using the statistical program IBM SPSS Statistics for Mac OS, version 28.

3. RESULTS

3.1. Hormones

The enzyme immunoassay used to quantify plasma aldosterone was validated for Kemp's ridley turtles by demonstrating close parallelism (*F*-test: $F_{1,9} = 1.07$, $p = 0.33$) and good accuracy (linear regression: $r^2 = 0.99$, slope = 0.84). Together, these tests indicate that the sample matrix did not interfere with antibody binding and that the assay could measure aldosterone across a range of concentrations with good mathematical accuracy (Fig. 1).

Summary statistics for initial and convalescent hormone data are provided in Table 1 and Fig. 2, and data for individual turtles are provided in Table S1. Initial concentrations of aldosterone and fT4 were not significantly different between lightly and heavily oiled turtles ($F_{1,28} = 0.02$, $p = 0.88$; $F_{1,26} = 1.87$, $p = 0.18$, respectively), while initial corticosterone concentrations were significantly different between oiling categories, being marginally greater in lightly oiled turtles ($F_{1,27} = 4.77$, $p = 0.04$). After successful rehabilitation, turtles exhibited significant changes in measured hormone concentrations. For both lightly and heavily oiled turtles, aldosterone and corticoster-

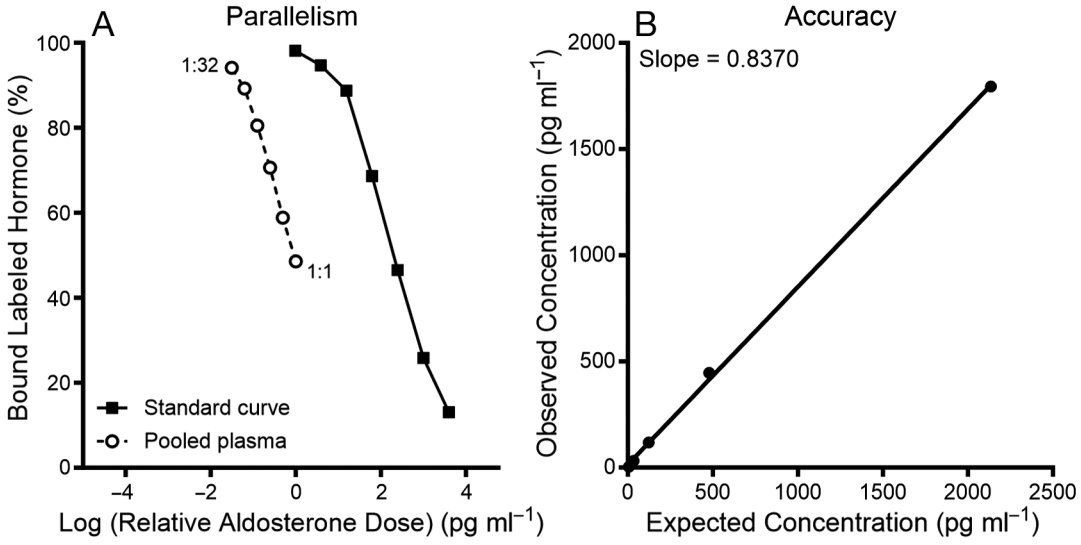

Fig. 1. Validation plots for measuring aldosterone in plasma extracts from Kemp's ridley turtles *Lepidochelys kempii* using enzyme immunoassay. (A) Close parallelism between serially diluted samples (1:1 to 1:32) to the aldosterone standard reference curve (8–5000 pg ml^{-1}). (B) Good accuracy demonstrated by the positive linear relationship of known aldosterone concentration (expected) against apparent concentration in spiked samples, with a slope between 0.8 and 1.0

one were lower ($F_{1,28}$ = 248.99, p < 0.001 and $F_{1,27}$ = 354.57, p < 0.001, respectively) and fT4 was higher in the convalescent state compared to initial concentrations ($F_{1,24}$ = 7.39, p = 0.012) (Table 1). The mean ± SD decrease in aldosterone was 540.6 ± 356.6 pg ml^{-1} (average 96% decrease), with convalescent values ranging from non-detectable to 37.4 pg ml^{-1}; the mean decrease in corticosterone concentration was 33.4 ± 14.3 ng ml^{-1} (average 90% decrease), with convalescent values ranging from 0.3 to 6.7 ng ml^{-1}; and the mean increase in fT4 was 1.2 ± 2.1 pg ml^{-1} (average 65% increase), with convalescent values ranging from non-detectable to 6.6 pg ml^{-1}. The effect sizes were large, with 61, 54, and 36% of the variability in corticosterone, aldosterone, and fT4 concentrations explained by temporal health state, respectively. Aldosterone was positively correlated with corticosterone, and negatively correlated with fT4 (Table 2). Comparative data for plasma aldosterone in various reptile species, corticosterone in *Lepidochelys kempii*, and fT4 in sea turtles are provided in Tables 3–5, respectively.

3.2. Plasma biochemistry

Summary statistics for initial and convalescent plasma biochemical data are provided in Table 1, and data for individual turtles are provided in Table S1. Correlations among plasma biochemical and endocrine data are provided in Table 2. Aldosterone was positively correlated with 4 of 9 biochemical analytes, including potassium, chloride, iCa, and lactate, and negatively correlated with sodium:potassium ratio. Corticosterone was positively correlated with potassium, chloride, iCa, and lactate, and negatively correlated with sodium:potassium ratio. Free T4 was not correlated with any biochemical analyte. Biochemical analytes also had a variety of correlations with each other.

4. DISCUSSION

This study is the first to validate a commercially available aldosterone assay for use in sea turtles, providing the largest data set to date for plasma aldosterone concentrations in sea turtles, and substantially adding to the limited data for plasma aldosterone in reptiles in general. This study also provides endocrine assessment of sea turtles affected by the DWH oil spill. The aldosterone assay that was validated in this study is a commercial enzyme immunoassay, making it readily accessible for use in most laboratory settings (i.e. does not involve radioactive isotopes), which may facilitate its use in future experimental and clinical studies of sea turtles and other reptiles.

Plasma aldosterone concentrations of vertebrates can vary widely. For example, an order of magnitude variation is seen within aldosterone reference intervals for both humans and dogs, with concentrations

Table 1. Summary of plasma hormone concentrations and biochemical analytes (mean ± SD; median and range in parentheses) of Kemp's ridley turtles *Lepidochelys kempii* affected by the *Deepwater Horizon* oil spill. Turtles represented 2 clinical groupings: lightly oiled and heavily oiled, as defined by Stacy (2015). Each turtle had 2 matched samples, one taken on the first day of hospitalization (initial) and a convalescent sample obtained later in the rehabilitation period (convalescence; >30 d later). Superscript letters denote significantly different groups ($p < 0.05$), according to repeated measures ANOVA. fT4: free thyroxine; iCa: ionized calcium; iMg: ionized magnesium; BUN: blood urea nitrogen; ND: non-detectable level. p-values in **bold** represent significant differences between groups

	Initial		Convalescence		Repeated measures ANOVA	
	Lightly oiled	Heavily oiled	Lightly oiled	Heavily oiled	Lightly vs. heavily oiled (initial values) p	Initial vs. convalescence p
Aldosterone (pg ml^{-1})	611 ± 372^{a} (519; 4.0–1264)	488 ± 340^{a} (342; 118–1196)	7.9 ± 9.1^{b} (5.6; ND–37)	9.5 ± 7.9^{b} (4.6; ND–29)	0.88	**<0.001**
Corticosterone (ng ml^{-1})	41 ± 11^{a} (44; 21–53)	32 ± 15^{b} (28; 16–64)	2.4 ± 1.7^{c} (2.1; 0.3–6.7)	2.9 ± 1.5^{c} (2.7; 0.9–6.1)	**0.04**	**<0.001**
fT4 (pg ml^{-1})	2.5 ± 0.7^{a} (2.3; 1.4–4.0)	2.2 ± 0.7^{a} (2.0; 1.5–3.8)	3.1 ± 2.1^{b} (2.7; ND–6.2)	3.7 ± 1.8^{b} (4.0; ND–6.6)	0.18	**0.012**
Na (mmol l^{-1})	145 ± 3.6 (144; 140–152)	146 ± 4.0 (146; 139–154)	146 ± 2.2 (145; 144–151)	145± 2.9 (145; 140–149)	0.62	0.94
K (mmol l^{-1})	4.8 ± 0.9^{a} (4.8; 3.6–6.0)	4.8 ± 0.9^{a} (4.5; 4.1–7.0)	3.4 ± 0.4^{b} (3.5; 2.8–4.0)	3.5 ± 0.4^{b} (3.4; 3.0–4.1)	0.45	**0.003**
Na: K ratio	31 ± 5.9^{a} (30; 24–39)	31 ± 4.6^{a} (33; 20–36)	44 ± 5.8^{b} (42; 36–53)	42 ± 4.1^{b} (42; 35–48)	0.39	**0.001**
Cl (mmol l^{-1})	122 ± 4.1 (122; 117–130)	121 ± 7.1 (122; 110–135)	117 ± 3.9 (117; 112–122)	117 ± 3.4 (115; 112–122)	0.41	0.06
iCa (mmol l^{-1})	0.95 ± 0.16 (0.96; 0.68–1.20)	0.75 ± 0.15 (0.75; 0.56–1.07)	0.64 ± 0.14 (0.69; 0.35–0.78)	0.70 ± 0.04 (0.70; 0.53–0.90)	0.33	0.06
iMg (mmol l^{-1})	1.55 ± 0.42^{a} (1.53; 0.70–2.17)	1.34 ± 0.48^{a} (1.22; 0.80–2.21)	1.27 ± 0.19^{b} (1.24; 1.00–1.52)	1.11 ± 0.19^{b} (1.08; 0.88–1.49)	0.32	**0.03**
Glucose (mg dl^{-1})	96 ± 52 (91; 34–196)	115 ± 64 (92; 48–219)	133 ± 28 (141; 76–164)	138 ± 11 (138; 118–154)	0.41	0.06
Lactate (mmol l^{-1})	5.1 ± 2.5^{a} (4.5; 2.0–9.8)	4.1 ± 2.1^{a} (3.8; 0.6–8.4)	1.4 ± 0.8^{b} (1.1; 0.7–3.3)	1.6 ± 0.5^{b} (1.7; 0.6–2.1)	0.81	**0.002**
BUN (mg dl^{-1})	75 ± 38^{a} (77; 31–155)	128 ± 45^{b} (130; 46–204)	70 ± 18^{a} (73; 39–101)	78 ± 31^{a} (70; 44–134)	**0.02**	0.09

generally ranging from the tens to low hundreds pg ml^{-1} (Meunier et al. 2015, Eisenhofer et al. 2017, Tan et al. 2020, Lin et al. 2021, Kai et al. 2022). Initial aldosterone concentrations in Kemp's ridley turtles in this study were substantially higher than maximal values within these mammalian reference intervals, and with 1 exception were higher than previously published data for aldosterone in reptiles, including limited data for 4 *Lepidochelys kempii* (Bradshaw & Grenot 1976, Uva et al. 1982, Balment & Loveridge 1989, Ortiz et al. 2000; Table 3). Among reptiles, only concentrations from the sea snake *Hydrophis cyanocinctus* were reported to be higher than those initially seen in the present study (with published sea snake concentrations being extreme outliers in comparison to other reptiles; Duggan & Lofts 1978). The average 96% decrease in plasma aldosterone concentrations of the DWH oil spill turtles after 30–59 d of convalescence likely reflects robust adrenal response followed by recovery from the initial stressors of oiling, capture, and transport, and acclimation to the rehabilitation facility. Indeed, convalescent aldosterone concentrations in this study were consistent with those of rehabilitated conspecifics during convalescence after cold-stunning (C. Innis unpubl. data). While this significant decline could be considered as possible evidence of post-oiling adrenal dysfunction, the clinical status of the convalescent turtles (clinically healthy, eating well, active, normalized plasma biochemical data), as well as similar values seen in non-oiled convalescent conspecifics do not support this interpretation. It is possible that the relatively high plasma aldosterone concentrations seen in previous reptile studies are due to an acute stress response under laboratory conditions, differences in methodology, or true natural variation among species.

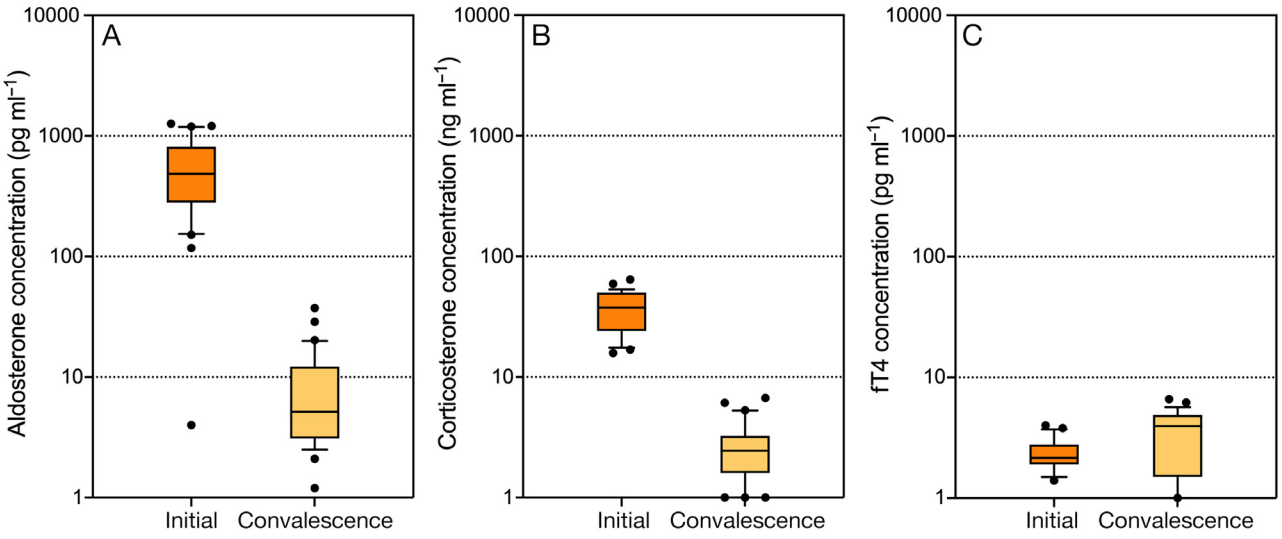

Fig. 2. Initial vs. convalescent plasma hormone concentrations (plotted on a logarithmic scale) for Kemp's ridley turtles affected by the *Deepwater Horizon* oil spill: (A) aldosterone; (B) corticosterone; (C) free thyroxine (fT4). For boxplots, the line inside the box indicates the median value, the height of the box encompasses the distance between the 25th and 75th percentiles, and the whiskers delineate 10th and 90th percentiles. Outlier points above and below the whiskers are marked with a circle. Initial hormone concentrations were significantly different from convalescent concentrations ($p < 0.05$)

Table 2. Correlation matrix for plasma hormone and biochemical measurements in Kemp's ridley turtles *Lepidochelys kempii* affected by oil exposure (n = 28 samples, except for fT4, n = 27). Values represent calculated Spearman's rank-order correlation coefficients (rho), as a measure of linear dependence between 2 variables (where rho = 1 is total positive correlation). Different color shades (heatmap) denote significant positive (shaded orange) and negative (shaded blue) correlations, with relative color intensities indicating more robust significance at $p < 0.05$ (*; lighter shades) and $p < 0.01$ (**; darker shades). fT4: free thyroxine; iCa: ionized calcium; iMg: ionized magnesium; BUN: blood urea nitrogen

	Aldosterone	Corticosterone	fT4	Na	K	Na:K ratio	Cl	iCa	iMg	Glucose	Lactate	BUN
Aldosterone	1											
Corticosterone	0.81**	1										
fT4	−0.40*	−0.40*	1									
Na	0.18	0.10	−0.09	1								
K	0.74**	0.70**	−0.20	0.11	1							
Na:K ratio	−0.74**	−0.71**	0.21	−0.05	−0.99**	1						
Cl	0.69**	0.52**	−0.27	0.45*	0.56**	−0.55**	1					
iCa	0.38*	0.51**	0.12	−0.08	0.58**	−0.60**	0.27	1				
iMg	0.34	0.30	0.04	0.20	0.38*	−0.38	0.54**	0.41*	1			
Glucose	−0.06	−0.19	0.13	0.30	−0.16	0.16	0.05	−0.32	0.13	1		
Lactate	0.75**	0.68**	−0.20	0.11	0.70**	−0.70**	0.41*	0.50**	0.48**	−0.08	1	
BUN	0.33	0.26	−0.08	0.54**	0.35	−0.33	0.37*	0.08	0.11	0.18	0.10	1

Like aldosterone, corticosterone concentrations of DWH oil spill turtles were initially high and then declined significantly with convalescence. Initial corticosterone concentrations were among the highest yet reported for this species, similar to initial concentrations for cold-stunned conspecifics (Hunt et al. 2012), and higher than those reported secondary to stressors such as nesting, gill net capture, entanglement net capture, and vehicle transport of 21–26 h duration (Gregory & Schmid 2001, Snoddy et al. 2009, Hunt et al. 2016a, 2019, 2020, Vásquez-Bultrón et al. 2021; Table 4). Convalescent corticosterone concentrations were very similar to those seen in conspecific nesting females and healthy conspecifics during convalescence from cold-stunning (Hunt et al. 2012, 2016a, 2019, 2020, Vásquez-Bultrón et al. 2021). Combined with these previous data, results of the present study suggest that baseline corticosterone concentrations for *L. kempii* are often ≤6 ng ml^{-1}, while severe stressors may elevate corticosterone into the range of 30–

Table 3. Comparative plasma aldosterone concentrations for reptiles under laboratory or hospital conditions (mean ± SE)

Species	Circumstance	Plasma aldosterone (pg ml^{-1})	Reference
North African spiny tailed lizard *Uromastyx acanthinura*	Laboratory baseline	360 ± 47.3	Bradshaw & Grenot (1976)
Shingleback skink *Tiliqua rugosa*	Laboratory baseline	317 ± 56	Bradshaw & Grenot (1976)
Kemp's ridley turtle *Lepidochelys kempii*	Laboratory baseline	351 ± 32	Ortiz et al. (2000)
	Oiled (hospital initial)	549.3 ± 64.9	This study
	Oiled (hospital convalescent)	8.7 ± 1.5	This study
Herman's tortoise *Testudo hermanni*	Laboratory baseline	82 ± 2	Uva et al. (1982)
Nile crocodile *Crocodylus niloticus*	Laboratory baseline	75 ± 12	Balment & Loveridge (1989)
Annulated sea snake *Hydrophis cyanocinctus*	Laboratory baseline	12700 ± 3400	Duggan & Lofts (1978)

Table 4. Comparative plasma corticosterone concentrations for Kemp's ridley turtles *Lepidochelys kempii* under various circumstances

Circumstance	Plasma corticosterone ng ml^{-1}	Reference
Lightly oiled (hospital initial)	41.4 ± 2.9 (mean ± SE)	This study
Heavily oiled (hospital initial)	32.1 ± 3.9 (mean ± SE)	This study
Cold-stunned (hospital initial)	39.3 ± 2.5 (mean ± SE)	Hunt et al. (2012)
Vehicle transport between hospital and release site	Means 11, 11, 19 after ~21–26 h transports	Hunt et al. (2016b, 2019, 2020)
Gill net capture (free range)	Mean 7.8; range 3.5–19.3	Snoddy et al. (2009)
Nesting (free range)	T0 min = 6.02 ± 3.21; T20 min = 8.20 ± 3.36; T40 min = 9.74 ± 4.26; T60 min = 12.22 ± 4.57 (mean ± SE)	Vásquez-Bultrón et al. (2021)
Entanglement net capture (free range)	T0 min = 6.16 ± 2.31; T30 min = 13.08 ± 3.53; T60 min = 24.68 ± 3.65 (mean ± SE)	Gregory & Schmid (2001)
Oiled (hospital convalescent)	2.7 ± 0.3 (mean ± SE)	This study
Cold-stunned (hospital convalescent)	<6	Hunt et al. (2012, 2016b, 2019, 2020)

Table 5. Comparative plasma free thyroxine (fT4) for sea turtles (mean ± SE). BLD: below limit of detection

Species	Circumstance	fT4, pg ml^{-1}	Reference
Kemp's ridley turtle *Lepidochelys kempii*	Oiled (hospital initial)	2.3 ± 0.1	This study
	Oiled (hospital convalescent)	3.4 ± 0.4	This study
	Cold-stunned (hospital initial)	Median BLD; range BLD–0.85	Hunt et al. (2012)
	Cold-stunned (hospital convalescent)	1.3 ± 1.5	Hunt et al. (2012)
Leatherback turtle *Dermochelys coriacea*	Hoop net capture (free range)	0.05 ± 0.05	Hunt et al. (2016b)
	Entangled or stranded (free range)	0.86 ± 0.37	Hunt et al. (2016b)

70 ng ml^{-1}. Although initial corticosterone values of lightly oiled turtles were statistically greater than those of heavily oiled turtles ($p = 0.04$), the magnitude of difference (difference of means = 9 ng ml^{-1}) is unlikely to be biologically or clinically important within the context of the very high overall values.

Free T4 concentrations in this study were relatively less variable than aldosterone and corticosterone. Although there was a significant increase in convalescent vs. initial concentrations, the change was more modest (mean increase of 65%). In comparison to limited fT4 data for sea turtles, in general, initial concentrations in DWH turtles were similar to those of convalescent cold-stunned conspecifics, and higher than those documented for leatherback turtles *Dermochelys coriacea* (Hunt et al. 2012, 2016b; Table 5). Convalescent fT4 concentrations in DWH turtles were also within the range described for convalescent cold-stunned conspecifics (Hunt et al. 2012, C. Innis unpubl. data).

A much larger biochemical data set from *L. kempii* that were evaluated during the DWH oil spill has been previously described (Stacy et al. 2017). Our study was not primarily designed to provide similarly broad biochemical evaluation given its relatively small sample size. Nonetheless, biochemical data in our study were consistent with findings of the larger data set, including evidence of initial physiologic derangement, and resolution of those derangements during convalescence (Table S1). The highest sodium, potassium, chloride, iCa, iMg, and lactate, and the highest and lowest glucose concentrations were seen in initial samples. Nine turtles had initial glucose concentrations consistent with hypo- or hyperglycemia (<50 or >150 mg dl^{-1}, respectively, including a range between 34 and 219 mg dl^{-1}), whereas only 2 convalescent turtles showed mild hyperglycemia (153, 164 mg dl^{-1}, respectively). Hypoglycemia may have been secondary to exertion, exhaustion, and anorexia. The highest plasma potassium concentrations were seen in initial samples (n = 18 turtles), including 9 turtles with plasma potassium greater than 5.0 mmol l^{-1}. All convalescent samples had potassium concentrations lower than 4.2 mmol l^{-1} (Table S1), and the sodium:potassium ratio increased because of these lower potassium concentrations. Elevated potassium concentrations are clinically concerning since hyperkalemia often carries a poor prognosis in hospitalized cold-stunned individuals of this species (Innis et al. 2009, Keller et al. 2012, Stacy et al. 2013). Initial BUN concentrations were significantly higher for heavily vs. lightly oiled turtles, which was the only biochemical difference detected between oiling categories. However, the absolute values for BUN are considered normal for this species, and the significant initial difference likely has minimal clinical relevance. In general, BUN concentrations of very ill or debilitated *L. kempii* tend to be low, which was not seen in this study. It is possible that this difference is an artifact of our relatively small sample size, the effect of outliers, or other clinically undetected differences that may affect BUN concentrations (e.g. hydration status, gastrointestinal ulceration with hemorrhage).

The correlations of hormone and biochemical data that were detected in our study do not necessarily indicate causal mechanisms, and some are likely due to coinciding adverse physiologic events. In some cases, these concurrent changes resulted in correlations that were not physiologically intuitive. For example, elevated aldosterone concentrations are typically expected to cause hypokalemia and hypernatremia, but in the present study, aldosterone was positively correlated with plasma potassium and not correlated with sodium. We believe that such unexpected correlations were the result of a concurrent suite of physiologic changes, including high corticosteroid concentrations and elevated concentrations of biochemical analytes that are related to dysregulation of cellular metabolism, acid–base balance (i.e. metabolic acidosis), renal function, and tissue perfusion (e.g. potassium, iCa, iMg, lactate). Further support for this concept was seen in corticosterone and glucose results. In general, and in several previous studies of this species, corticosterone is expected to be positively correlated with glucose (Hunt et al. 2012, 2016a, 2019, 2020). In the present study, corticosterone was not correlated with glucose concentrations, likely due to the influence of various factors resulting in highly variable initial glucose concentrations, which were often very low.

Many studies have examined the effects of petroleum exposure on the adrenal glands and the hypothalamic–pituitary–adrenal axis of animals using a variety of petroleum types, dosing regimens (including natural and experimental exposures), and methods of evaluation. Outcomes range from no observed changes (Beckett et al. 2002, Horak et al. 2017), to nonspecific adrenal hypertrophy (Mohr et al. 2008, 2010, Bursian et al. 2017), to relative decreases in adrenocortical responsiveness (Gorsline & Holmes 1982b, Lattin et al. 2014, Schwacke et al. 2014). This disparity may reflect considerable differences in methodology as well as potential variation in species susceptibility. Most of these studies entailed longer oil exposures of weeks to months; however, adrenal effects have been

described in birds exposed to oil for as little as 10 d or less (Miller et al. 1978, Gorsline & Holmes 1982a,c, Leighton 1986). The dose and duration of oil exposure was unknown for the sea turtles that were included in our study, but based on necropsies of dead oiled turtles, live turtles that were heavily exposed likely had extensive oiling of the esophagus that persisted even after oil was cleaned from external surfaces. An oily sheen was noted for up to 1 wk when heavily oiled live turtles defecated, suggesting at least a modest duration of internal oil exposure (B. Stacy unpubl. data). While results of our study support previous investigations that described significant physiologic derangements in oiled sea turtles that were rescued during the DWH spill (Stacy et al. 2017), our findings do not indicate adrenal dysfunction. We cannot, however, exclude the possibility of adverse adrenal effects in sea turtles associated with more chronic oil exposures.

There are several limitations of our study. Methodologically, like other retrospective clinical endocrine studies of this species (Hunt et al. 2012), and unlike well controlled prospective endocrine studies in this species (Hunt et al. 2016a, 2019, 2020), the time of blood sample collection relative to handling was not recorded, which could have resulted in increased corticosteroid and glucose concentrations if prolonged. The present study does not allow differentiation of potential causal mechanisms since all turtles were affected by serial events, including exposure to oil, thermal extremes of unknown severity and duration, capture, and transportation (Hunt et al. 2012, Stacy et al. 2017). Further, samples were only evaluated from 2 of the 4 oiling categories that defined these turtles in previous studies (i.e. we did not evaluate turtles previously defined as 'minimally oiled' or 'moderately oiled'). As such, this study may have failed to detect changes and correlations that may have been seen if a larger, more representative sample set were evaluated. Finally, although samples utilized in this study had been frozen for approximately 11 yr, and research on long-term stability (years) of plasma samples for hormone and other chemistry analytes is limited, it does appear that corticosteroid and thyroid hormones are relatively tolerant of long-term freezing and freeze–thaw cycles (Kley & Rick 1984, Männistö et al. 2007, Lie & Thorstensen 2018); and the observed biochemical data were typical of the species, including previous data for this cohort that were not subject to long-term storage (Innis et al. 2009, Keller et al. 2012, Stacy & Innis 2015, Hunt et al. 2016b, 2019, 2020, Stacy et al. 2017). The samples used in this study were collected, processed, and stored similarly; thus, even if storage may have affected absolute analyte concentrations, the relative data are considered comparable among oiling categories and at both time points.

In summary, our study provides additional insight into the physiologic status of *L. kempii* that were affected by the DWH oil spill. We provide comparative data and a validated method for further evaluation of aldosterone in clinical and experimental studies of sea turtles. Given the potential physiologic influence of elevated corticosteroid concentrations, greater understanding of the role and influence of aldosterone in the acute stress response and health of endangered sea turtle species is needed. Aldosterone appears to have a key function in the stress response of marine vertebrates studied to date (Atkinson et al. 2015) and may serve as a highly useful and sensitive indicator of stress in wildlife in a changing ocean.

Acknowledgements. Morris Animal Foundation funded this study (MAF Project D20ZO-090). Deana Edmunds, New England Aquarium, provided technical laboratory assistance. The findings and conclusions in this presentation are those of the authors and do not necessarily represent the view of the National Oceanic and Atmospheric Administration and the US Department of the Interior or of any other natural resource Trustees for the BP/*Deepwater Horizon* Natural Resources Damage Assessment (NRDA). We thank all volunteers, veterinarians, technicians, animal care staff, field responders, and government agency personnel who participated in the rescue and rehabilitation of sea turtles during the *Deepwater Horizon* oil spill. Rehabilitation facilities included Audubon Nature Institute (New Orleans, LA), Gulf World Marine Park (Panama City, FL), Institute for Marine Mammal Studies (Gulfport, MS), Clearwater Marine Aquarium (Clearwater, FL), Disney Animal Kingdom (Lake Buena Vista, FL), Florida Aquarium (Tampa, FL), Gulfarium (Fort Walton Beach, FL), Mote Marine Laboratory and Aquarium (Sarasota, FL), and SeaWorld (Orlando, FL). Special thanks to C. Field, L. Staggs, R. MacLean, J. Keene, D. Cacela, C. Pelton, C. Cray, M. Kelley, S. Holmes, S. Boylan, C. Harms, T. Norton, G. Lewbart, C. Chevis, M. Piscitelli, C. Merigo, A. Tuttle, K. Sampson, and M. Ziccardi. Veterinarians, technicians, biologists, and other vital personnel from throughout the country supported these facilities and were essential to response efforts. All activities and samples described herein were conducted and collected with authorization of the United States Fish and Wildlife Service and National Oceanic and Atmospheric Administration.

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Editorial responsibility: Richard Reina, Clayton, Victoria, Australia
Reviewed by: C. Ullmer and C. Champagne

Submitted: October 21, 2023
Accepted: February 20, 2024
Proofs received from author(s): April 16, 2024