BASELINE PLASMA THROMBOELASTOGRAPHY IN KEMP'S RIDLEY (LEPIDOCHELYS KEMPII), GREEN (CHELONIA MYDAS) AND LOGGERHEAD (CARETTA CARETTA) SEA TURTLES AND ITS USE TO DIAGNOSE COAGULOPATHIES IN COLD-STUNNED KEMP'S RIDLEY AND GREEN SEA TURTLES

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Source: Journal of Zoo and Wildlife Medicine, 50(1) : 62-68

Published By: American Association of Zoo Veterinarians

URL: https://doi.org/10.1638/2018-0142

Journal of Zoo and Wildlife Medicine 50(1): 62–68, 2019

BASELINE PLASMA THROMBOELASTOGRAPHY IN KEMP'S RIDLEY (*LEPIDOCHELYS KEMPII*), GREEN (*CHELONIA MYDAS*) AND LOGGERHEAD (*CARETTA CARETTA*) SEA TURTLES AND ITS USE TO DIAGNOSE COAGULOPATHIES IN COLD-STUNNED KEMP'S RIDLEY AND GREEN SEA TURTLES

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Abstract: Cold-stunning in sea turtles is a frequent natural cause of mortality and is defined as a hypothermic state due to exposure to water temperatures <12°C. Derangements of biochemistry and hematology data by cold stunning have been well documented, although the effects on coagulation have not yet been investigated. The objectives of this study were to characterize the hemostatic state of non–cold-stunned sea turtles and to compare cold-stunned sea turtles at admission and after successful rehabilitation via a sea turtle–specific thromboelastography (TEG) protocol. TEG enables evaluation of the entire coagulation process, and the methodology has recently been established in sea turtles. Initially, 30 wild and apparently healthy sea turtles were sampled as controls: loggerhead sea turtles (*Caretta caretta*), $n = 17$; Kemp's ridley sea turtles (*Lepidochelys kempii*), $n = 8$; and green turtles (*Chelonia mydas*), $n = 5$. In addition, paired TEG samples were performed on 32 *Ch. mydas* and 14 *L. kempii* at admission and prerelease after successful rehabilitation from cold stunning. Statistically significant differences in reaction time, kinetics, angle, and maximum amplitude parameters in *L. kempii* and *Ch. mydas* species demonstrated that the time taken for blood clot formation was prolonged and the strength of the clot formed was reduced by cold stunning. These findings indicate that cold stunning may cause disorders in hemostasis that can contribute to the severity of the condition. Early diagnosis of coagulopathies in the clinical assessment of a cold-stunned sea turtle may influence the treatment approach and clinical outcome of the case.

Key words: Coagulation, coagulopathy, cold stunned, sea turtle, thromboelastography.

INTRODUCTION

Cold stunning is the term used to describe the effects of hypothermic environmental conditions on sea turtles, resulting in lethargic-to-moribund clinical states.[16] It is defined as a hypothermic state due to exposure to cold water temperatures <12°C.[5,21,26] Reports indicate cold-stunning affects Kemp's ridley sea turtles (*Lepidochelys kempii*), green turtles (*Chelonia mydas*), and loggerhead sea turtles (*Caretta caretta*).[1,5,6,15,17–19,32] These three species comprise the vast majority of the marine turtle population of the east coast of the Unites States.[10] Recent large-scale mortalities, particularly in *L. kempii*, have been attributed to cold stunning.[16,18] Juvenile sea turtles are the most affected life stage, usually due to failure to migrate to warmer waters in late fall after foraging in shallow waters of the Northwest Atlantic.[17] Chronic cold-stunning events typically occur in higher latitudes, e.g., New England, with exposure to cold temperatures lasting for more than a 2-wk period. Acute cold-stunning events typically occur over a short time frame, e.g., 1 or 2 wk and occur at lower latitudes, e.g., NC.[16] Several studies have identified hematological, biochemical, and

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acid-base changes in cold-stunned turtles, but to the authors' knowledge, hemostatic disorders have not been clinically assessed.[1,5,17,18]

Thromboelastography (TEG) remains relatively unexplored in reptiles due to differences from mammalian coagulation factors affecting in vitro clotting in this taxon. The lack of the intrinsic system and factors XI and XII are unique features in reptiles in comparison to mammals.[20,30,31] TEG is the only coagulation test to provide global evaluation of the hemostatic process rather than focusing on a specific pathway, such as in the routinely used prothrombin and activated partial thromboplastin tests.[8] TEG focuses on four main parameters: reaction time (R), clot formation time (K), clot formation rate [angle (α)], and maximum amplitude (MA), which indicates clot strength.[8,13,36] Recent investigations using TEG have highlighted the effects of hypothermia resulting in coagulopathies as part of cold stress syndrome in the Florida manatee (*Trichechus manatus latirostris*).[3] Clinical and pathological findings of disseminated intravascular coagulation (DIC) and hyphema in a small number of cold-stunned sea turtles a few days after initial recovery have been reported previously.[16] The objectives of this study were to determine baseline TEG values in three species of sea turtles and to characterize the hemostasis of cold-stunned sea turtles at admission and after successful rehabilitation via a sea turtle–specific TEG protocol.

MATERIALS AND METHODS

Cold-stunned turtles admitted to New England Aquarium (NEAQ) in winters 2015 and 2016 were sampled as part of their admission exam (*L. kempii*, $n = 20$; *Ch. mydas*, $n = 13$). Venous blood was collected from the dorsal cervical sinus by standard blood sampling techniques,[22] into a 3-ml unheparinized syringe and transferred into a 0.32% sodium citrate tube with appropriate required filling volume of whole blood (MiniCollect sodium citrate, Greiner Bio-One, Kremsmünster, 4550, Austria). Study inclusion criteria were a body weight greater than 1 kg and admission packed cell volume (PCV) greater than 24%. Turtles either remained at NEAQ for rehabilitation or were transferred to the GA Sea Turtle Center, the Center for Marine Sciences and Technology (CMAST), the Karen Beasley Sea Turtle Rescue and Rehabilitation Center (KBSTRRC), or the NC Aquarium at Pine Knoll Shores. A second sample was obtained from each sea turtle after successful rehabilitation to obtain paired samples. Additional cold-stunned samples (*Ch. mydas*, $n = 28$ total, 21 paired) were obtained from cases admitted directly to CMAST, KBSTRRC, and the Sea Turtle Assistance and Rehabilitation (STAR) Center at the NC Aquarium on Roanoke Island, which remained there for rehabilitation and were resampled prerelease. The cold-stunned *Ch. mydas* in NC are considered a more acute presentation than those in New England.[16]

In addition, during September 2015 and September 2016 wild sea turtle captures were carried out by National Oceanographic and Atmospheric Administration biologists in conjunction with CMAST in NC. Turtles were captured using a pound net in Core Sound near Harkers Island, NC (~34.6°N, –076.5°W) as described previously.[9,14] *Caretta caretta*, *L. kempii*, and *Ch. mydas* sea turtles were sampled to assess normal wild sea turtle coagulation (*C. caretta*, $n = 16$; *L. kempii*, $n = 8$; *Ch. Mydas*, $n = 5$).

All whole blood samples were kept insulated in a cool box during transport to the laboratory for processing. Whole blood was centrifuged at 800 *g* with an IDEXX Statspin® centrifuge (IDEXX, Westbrook, ME 04092, USA) for 6 min to obtain plasma. Plasma was frozen at –80°C, and samples were shipped to NC State University for TEG analysis. For each sample, TEG was performed according to a recently published sea turtle–specific methodology,[4] via two TEG 5000 machines (TEG® 5000 hemostasis analyzer, Haemonetics Corporation, Braintree, MA 02184, USA), each with two channels. As reptiles are poikilothermic, the operating temperature of both machines was reduced to 30°C instead of the standard 37°C as used for mammalian samples. Thromboplastin was obtained from a pooled sample of sea turtle brain. The protocol requires 10 μl of frozen sea turtle brain thromboplastin to be added to 20 μl of $CaCl_2$ and 340 μl of plasma. After thawing, plasma samples were allowed to sit at room temperature for 30 min, and channel selection was randomized. Preinstalled software (TEG Analytical Software 4.2.3, Haemonetics Corporation) was used to generate and capture variables for the TEG tracing.

Statistical analysis was performed on the paired samples to assess the effects of cold stunning on admission values. Before test selection, data were assessed for normality by the Kolmogorov–Smirnov test, and subsequently nonparametric tests were used throughout statistical analysis. Wilcoxon signed rank tests were used to compare cold-stunned samples with prerelease samples. Comparison was also performed between *Ch. mydas*

Table 1. Baseline data for plasma thromboelastography on normal wild green turtles (*Chelonia mydas*), Kemp's ridley sea turtles (*Lepidochelys kempii*), and loggerhead sea turtles (*Caretta caretta*), with samples consisting of prerelease rehabilitated turtles in addition to wild caught turtles for *Ch. mydas* and *L. kempii*.

Species	Component[a]	Mean	Median	SD	Minimum	Maximum
Ch. mydas, $n = 37$	R (min)	1.50	1.20	0.67	0.8	3.2
	K (min)	0.96	0.80	0.84	0.8	5.8
	α (°)	80.38	82.30	6.62	56.8	85.5
	MA (mm)	37.92	36.70	9.09	9.5	55.7
L. kempii, $n = 24$	R (min)	2.38	2.50	0.98	1.1	4.3
	K (min)	0.84	0.80	0.20	0.8	1.8
	α (°)	82.4	83.45	4.56	66.3	86.7
	MA (mm)	55.1	59.30	13.24	30.9	79.7
C. caretta, $n = 17$	R (min)	1.9	1.40	1.46	0.8	6.6
	K (min)	0.8	0.8	0.34	0.8	2.2
	α (°)	80.1	82.8	6.46	58.2	85.6
	MA (mm)	44.6	42.7	10.69	27.6	69.0

[a] R indicates reaction time; K, clot formation time; α, clot formation rate; MA, maximum amplitude.

turtles admitted to NEAQ ($n = 13$) with *Ch. mydas* turtles admitted in NC ($n = 28$) to assess for possible differences between chronic (NEAQ) vs acute (NC) cold-stunning presentations. A Mann-Whitney U-test was used to compare these two groups. All analyses were performed using commercially available statistical software program R (www.R-project.org), with significance set at $P < 0.05$.

RESULTS

Baseline mean, SD, median, and range for TEG data from all three species are presented in Table 1. Prerelease results from *Ch. mydas* and *L. kempii* were combined with results from healthy free-ranging turtles for baseline values. Paired citrated plasma samples (cold-stunned admission and postrehabilitation) were obtained for 32 *Ch. mydas* and 14 *L. kempii*. Comparison of cold-stunned admission samples with prerelease samples revealed statistically significant differences in both *Ch. mydas* (Table 2) and *L. kempii* (Table 3). The *P*-values showed significantly slower α in cold-stunned individuals, with $P = 0.0016$ in *Ch. mydas* and $P = 0.00107$ in *L. kempii*. The MA was also significantly lower at the time of cold stunning in both species, with $P = 0.00034$ and $P = 0.00032$ in *Ch. mydas* and *L. kempii*, respectively.

Two *L. kempii* and one *Ch. mydas* that were severely debilitated died shortly after admission sampling and therefore were not available for paired sampling prerelease. In comparison of their data with the average data for the respective species, they demonstrated severe abnormalities with a markedly prolonged R and reduced α and MA, that may have served as a marker for severity of disease, although some survivors had similarly low values for α and MA. The contrasting thromboelastograms, compared to a normal *Ch. mydas* turtle, are demonstrated in Figure 1. Insufficient numbers prevented statistical analysis relative to the cases which survived.

Sample comparison between the NEAQ "chronically" cold-stunned *Ch. mydas* turtles and NC "acutely" cold-stunned *Ch. mydas* turtles did not demonstrate statistically significant differenc-

Table 2. Paired admission and prerelease plasma thromboelastography values for cold-stunned and rehabilitated green turtles ($n = 32$ except for clot formation time, where $n = 27$; Wilcoxon matched pairs signed rank test). Values in bold indicate significance $P < 0.05$.

	Admission		Prerelease		
TEG measurement[a]	Mean (SD)	Median (10th, 90th percentile)	Mean (SD)	Median (10th, 90th percentile)	*P*
R (min)	1.57 (0.82)	1.25 (0.83, 2.96)	1.54 (0.70)	1.30 (0.8, 2.77)	0.39
K (min)	1.69 (2.09)	0.80 (0.80, 5.02)	1.01 (0.97)	0.80 (0.80, 0.96)	**0.0139**
α (°)	73.2 (11.5)	77.8 (57.5, 82.1)	80.0 (7.0)	82.3 (64.7, 85.2)	**0.0016**
MA (mm)	27.7 (12.7)	26.8 (12.7, 38.3)	37.7 (9.5)	36.4 (28.8, 52.7)	**0.00034**

[a] TEG indicates thromboelastography; R, reaction time; K, clot formation time; α, clot formation rate; MA, maximum amplitude.

Table 3. Paired admission and prerelease plasma thromboelastography values for cold-stunned and rehabilitated Kemp's ridleys ($n = 14$ except for clot formation time, where n = 12; Wilcoxon matched pairs signed rank test).

	Admission		Prerelease		
TEG measurement[a]	Mean (SD)	Median (10th, 90th percentile)	Mean (SD)	Median (10th, 90th percentile)	*P*
R (min)	4.79 (3.36)	3.90 (1.55, 11.15)	2.91 (0.83)	2.80 (1.65, 4.25)	0.158
K (min)	1.43 (0.82)	1.10 (0.80, 2.94)	0.87 (0.27)	0.80 (0.80, 1.30)	n/s[b]
α (°)	67.8 (14.1)	74.7 (42.7, 80.5)	81.0 (5.5)	82.4 (70.7, 86.4)	**0.00107**
MA (mm)	30.4 (8.4)	30.0 (13.4, 42.4)	62.5 (8.7)	62.4 (49.8, 76.8)	**0.00032**

[a] TEG indicates thromboelastography; R, reaction time; K, clot formation time; α, clot formation rate; MA, maximum amplitude.

[b] Too many tied pairs to compute a *P*-value. As the statistical test is a matched pairs test if the majority of the pairs are identical (such as in K) then a *P* value cannot be computed.

es (Table 4). Clot strength or MA showed the greatest difference between the two groups, with $P = 0.058$.

DISCUSSION

This is the first report of the use of a species-specific TEG protocol in cold- stunned sea turtles. As an investigational technique, it is premature for use in establishing formal reference intervals or making cross-species comparisons of clotting parameters. It does however effectively demonstrate clear differences in blood coagulation between normal and cold-stunned sea turtles within species. Cold stunning in sea turtles has been well reported to affect biochemistry, hematology, and blood gas analysis; however, coagulopathies have not been previously confirmed via laboratory testing.[6,17,32] Clinical observations consistent with coagulopathies have been described in cold-stunned turtles with hyphema and other signs typically associated with DIC such as intracoelomic hemorrhage, plummeting PCV, and histopathology findings.[16] Comparison of TEG results between admission and prerelease demonstrated a significantly reduced α and MA in cold-stunned animals compared with the time of release. *Chelonia mydas* turtles also showed significant differences in R and K, demonstrating that the time to initiate clot formation is also affected by cold stunning; however, this may be more species specific. It is possible that this acquired coagulopathy is adaptive in extreme cold conditions and serves to maintain microcirculation by inhibiting microthrombi formation, but at the expense of the potential for increased bleeding tendency.[12,23] Effects of hypothermia on coagulation are well documented in humans and dogs, with core temperatures below 35°C resulting in platelet dysfunction, decreased plasminogen activation, and delayed thrombus formation.[23,25,27,28,33–35,37] Statistically significant effects of hypothermia in mammals, including a delay of thrombus initiation at 24°C, occurred at an approximately 36% decrease in core temperature.[28] Admittance temperatures of the sea turtles were as low as 1.6°C (Fig. 2), indicating a much greater proportionate reduction in normal functional core body temperature; therefore, it is possible the coagulation parameters would be affected by this degree of hypothermia. Although turtles are poikilothermic and would naturally sustain large temperature fluctuations, hypothermia does appear to affect coagulation, as demonstrated by these results.

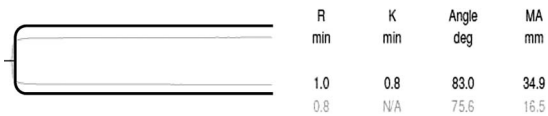

Figure 1. Thromboelastograms of a cold-stunned green turtle that died (light grey line) in comparison with normal green turtle (black line). Note the marked difference in clot strength. K is N/A as MA was <20 mm which is the predetermined value to obtain K.

Despite there being no statistically significant differences between admission data of NEAQ (chronic) and NC (acute) cold-stunned *Ch. mydas* turtles (Table 4), there were suggestive differences in the thromboelastograms produced, with chronic cases showing longer time to reach maximum clot strength and acute cases achieving less overall blood clot strength. There were significantly different values in K, α, and MA in comparison with normal *Ch. mydas* turtles for both acute and chronic cases (Table 2). These results indicate that both acute and chronic cold stunning has similar effects on green turtle (*C.*

Table 4. Comparison of thromboelastography values for cold-stunned green turtle admitted at New England Aquarium ($n = 13$) to those admitted in NC ($n = 28$) (Mann-Whitney U-test, on two unpaired groups).

	NEAQ[b]		NC		
TEG measurement[a]	Mean (SD)	Median (10th, 90th percentile)	Mean (SD)	Median (10th, 90th percentile)	*P*
R (min)	1.78 (0.95)	1.3 (0.8, 3.6)	1.52 (0.84)	1.25 (0.80, 3.26)	0.459
K (min)	2.02 (2.29)	1.15 (0.80, 7.28)	1.35 (1.70)	0.80 (0.80, 2.52)	0.076
α (°)	71.28 (13.1)	71.15 (45.34, 82.68)	72.27 (12.77)	77.85 (54.21, 81.81)	0.0696
MA (mm)	32.25 (15.74)	28.9 (17.98, 63.34)	23.31 (9.78)	25.3 (10.0, 34.03)	0.0587

[a] TEG indicates thromboelastography; R, reaction time; K, clot formation time; α, clot formation rate; MA, maximum amplitude.
[b] NEAQ indicates New England Aquarium.

mydas) clotting capabilities. Further research is needed to tease out whether the time taken to return to a normal coagulation status is affected by the duration of cold stunning. In addition, as the TEG was performed using plasma instead of whole blood, the effects of thrombocytes cannot be assessed, only coagulation factors and functional fibrinogen.[7] Performing whole blood TEG would enable a more holistic analysis; however, it is practically more challenging when working with wild species, leading to the choice of plasma in this study. As TEG machines are not readily available in veterinary medicine, providing a methodology to use plasma instead of whole blood enables freezing of plasma to allow delayed processing in comparison to the use of whole blood, which requires immediate analysis. Sample results were not affected after a period of prolonged storage (1 yr at −80°C), thereby allowing frozen/archived samples to be used.[4]

Disseminated intravascular coagulation can be triggered by any disease process that decreases endogenous anticoagulants, increases prothrombotic factors, causes fibrinolysis, or causes endothelial dysfunction.[24] Identifying the effects of hypothermia on coagulation in sea turtles may have potential in rapidly diagnosing developing DIC. It is well documented in mammalian species that one of the concurrent changes when DIC is present is thrombocytopenia.[2,24,29] A reduction in thrombocytes has also been associated with disease in sea turtles,[11] and anemia has been confirmed as one of the clinicopathological changes that occurs during cold-stunning events.[15] As this study used plasma TEGs, the effects of thrombocytopenia due to hypothermia could not be quantified. In addition, a fatal hemolytic syndrome has been documented by clinicians at NC, the New England Aquarium, and the GA Sea Turtle Center.[16] Necropsy and histopathological findings include diffuse intracoelomic hemorrhage and multiorgan intravascular thrombosis, respectively (Harms, unpublished data). Further research and exploration of coagulopathies in cold-stunned sea turtles, including fibrinogen concentrations and D-dimer validation, may determine the extent of DIC in these species.

Figure 2. Hypothermia on arrival with core temperature 1.9°C. Photo credit Dr. Craig Harms.

Based on the stark differences observed in TEG values for cases that did not survive (Fig. 1), performing TEG at the time of admission could provide the clinician with useful information for treatment and monitoring and potentially improve rehabilitation outcome. For example, observing a very abnormal TEG in conjunction with poor clinical signs and low PCV may encourage more extensive therapy such as a whole blood transfusion rather than continued monitoring. There were however other individuals with abnormal TEG values as severe as the non-survivors that did survive. They demonstrated a marked improvement in their TEGs at the time of release. Lack of change or improvement over time in successive TEGs may be more indicative of a poor prognosis than a single TEG. Successive TEGs that demonstrate deterioration and progressive

increase in LY30 (increased fibrinolysis) could be candidates for antifibrinolytic therapy such as aminocaproic acid; however, this has not been documented in reptiles.

Further research is required to establish species-specific reference intervals and to determine survival thresholds. This study uses TEG as an effective investigational diagnostic tool to identify coagulopathies in cold-stunned sea turtles and highlights the severity of the condition. By characterizing the effects of cold-stunning this study adds to considerations for treatment protocols and improves understanding of the pathophysiology of this condition.

Acknowledgments: The authors thank the Rescue and Rehabilitation and Animal Health Departments at New England Aquarium for sampling assistance and sea turtle care during rehabilitation and the staff at KBSTRRC, STAR, and NC Aquarium at Pine Knoll Shores for dedicated care during rehabilitation. Also, thanks to Dr. Terry Norton at the GA Sea Turtle Center for turtle care and assistance with prerelease sample collection. In addition, the authors thank Joanne Braun McNeill, Larisa Avens, and April Hall for sample collection from free-ranging turtles and Heather Broadhurst, Matthew Godfrey, and Sarah Finn for collection of brain tissue. Samples were collected under National Marine Fisheries Service Endangered Species Act scientific research permit 16733 and held and processed under NC Wildlife Resources Commission Endangered Species permits 15ST44 and 16ST42. NEAQ samples were collected under US Fish and Wildlife permit TE-697823 and NEAQ Animal Care and Use Committee protocol 2015-25.

LITERATURE CITED

1. Anderson ET, Harms CA, Stringer EM, Cluse WM. Evaluation of hematology and serum biochemistry of cold-stunned green sea turtles (*Chelonia mydas*) in North Carolina, U.S.A. J Zoo Wildl Med. 2011;42(2): 247–255.

2. Barratclough A, Ball R, Francis-Floyd R, Reep R, Conner B. Identifying disseminated intravascular coagulation in the Florida manatee (*Trichechus manatus latirostris*) and understanding its clinical implications J Zoo Wildl Med. 2017;1(48):152–158.

3. Barratclough A, Conner BJ, Brooks MB, Stablein AP, Gerlach TJ, Reep RL, Ball RL, Floyd RF. Identifying coagulopathies in the pathophysiology of cold stress syndrome in the Florida manatee (*Trichechus manatus latirostris*). Dis Aquat Organ. 2017;125(3): 179–188.

4. Barratclough A, Hanel R, Stacy N, Ruterbories L, Christiansen E, Harms C. Establishing a protocol for thromboelastography in sea turtles. Vet Rec. 2018;5(1): e000240.

5. Burke VJ, Standora EA, Morreale SJ. Factors affecting strandings of cold-stunned juvenile Kemp's ridley and loggerhead sea turtles in Long Island, New York. Copeia. 1991(4):1136–1138.

6. Carminati C, Gerle E, Kiehn L, Pisciotta R. Blood chemistry comparison of healthy vs hypothermic juvenile Kemp's ridley sea turtles (*Lepidochelys kempi*) in the New York Bight. In: Proceedings of the fourteenth annual symposium on sea turtle biology and conservation. National Oceanic and Atmospheric Administration (NOAA) technical memorandum NMFS-SEFSC-351. Miami (FL): NOAA; 1994. P. 203–207.

7. Chitlur M, Sorensen B, Rivard G, Young G, Ingerslev J, Othman M, Nugent D, Kenet G, Escobar M, Lusher J. Standardization of thromboelastography: a report from the TEG-ROTEM working group. Haemophilia. 2011;17(3):532–537.

8. Donahue SM, Otto CM. Thromboelastography: a tool for measuring hypercoagulability, hypocoagulability, and fibrinolysis. J Vet Emerg Crit Care. 2005;15(1): 9–16.

9. Epperly SP, Braun-McNeill J, Richards PM. Trends in catch rates of sea turtles in North Carolina, USA. Endang Spec Res. 2007;3(3):283–293.

10. Epperly SP, Braun J, Veishlow A. Sea turtles in North Carolina waters. Conserv Biol. 1995;9(2):384–394.

11. Flint M, Morton JM, Limpus CJ, Patterson-Kane JC, Murray PJ, Mills PC. Development and application of biochemical and haematological reference intervals to identify unhealthy green sea turtles (*Chelonia mydas*). Vet J. 2010;185(3):299–304.

12. Forman KR, Wong E, Gallagher M, McCarter R, Luban NLC, Massaro AN. Effect of temperature on thromboelastography and implications for clinical use in newborns undergoing therapeutic hypothermia. Pediatr Res. 2014;75(5):663–669.

13. Goggs R, Wiinberg B. Variability in veterinary thromboelastography. J Vet Emerg Crit Care. 2012; 22(2):145–147.

14. Harms CA, Mallo KM, Ross PM, Segars A. Venous blood gases and lactates of wild loggerhead sea turtles (*Caretta caretta*) following two capture techniques. J Wild Dis. 2003;39(2):366–374.

15. Innis C, Nyaoke AC, Williams III CR, Dunnigan B, Merigo C, Woodward DL, Weber ES, Frasca S Jr. Pathologic and parasitologic findings of cold-stunned Kemp's ridley sea turtles (*Lepidochelys kempii*) stranded on Cape Cod, Massachusetts, 2001–2006. J Wild Dis. 2009;45(3):594–610.

16. Innis CJ, Staggs LA. Cold Stunning. In: Manire CA, Norton TM, Stacy B, Harms CA, and Innis CJ

(eds.). Sea turtle health and rehabilitation. Plantation (FL): J. Ross Publishing, Inc; 2017. p. 675–687.

17. Innis CJ, Tlusty M, Merigo C, Weber ES. Metabolic and respiratory status of cold-stunned Kemp's ridley sea turtles (*Lepidochelys kempii*). J Comp Physiol B 2007;177(6):623–630.

18. Keller KA, Innis CJ, Tlusty MF, Kennedy AE, Bean SB, Cavin JM, Merigo C. Metabolic and respiratory derangements associated with death in cold-stunned Kemp's ridley turtles (*Lepidochelys kempii*): 32 cases (2005–2009). J Am Vet Med Assoc. 2012; 240(3):317–323.

19. Kelly TR, McNeill JB, Avens L, Hall AG, Goshe LR, Hohn AA, Godfrey MH, Mihnovets AN, Cluse WM, Harms CA. Clinical pathology reference intervals for an in-water population of juvenile loggerhead sea turtles (*Caretta caretta*) in Core Sound, North Carolina, USA. PLOS ONE 2015;10(3):e0115739.

20. Lewis JH. Comparative hemostasis. In: Comparative hemostasis in vertebrates. Pittsburgh (PA): Springer; 1996. p. 325–359.

21. Morreale SJ, Meylan AB, Sadove SS, Standora EA. Annual occurrence and winter mortality of marine turtles in New York waters. J Herpetol. 1992:301–308.

22. National Marine Fisheries Service (NMFS). Sea turtle research techniques manual. NOAA Technical Memorandum NMFS-SEFSC 2008;579.

23. Polderman KH. Hypothermia and coagulation. Crit Care. 2012;16(2):A20.

24. Ralph AG, Brainard BM. Update on disseminated intravascular coagulation: when to consider it, when to expect it, when to treat it. Top Companion Anim Med. 2012;27(2):65–72.

25. Reed R, Bracey A Jr, Hudson J, Miller T, Fischer R. Hypothermia and blood coagulation: dissociation between enzyme activity and clotting factor levels. Circ Shock 1990;32(2):141–152.

26. Roberts K, Collins J, Paxton CH, Hardy R, Downs J. Weather patterns associated with green turtle hypothermic stunning events in St. Joseph Bay and Mosquito Lagoon, Florida. Phys Geogr. 2014;35(2): 134–150.

27. Rohrer MJ, Natale AM. Effect of hypothermia on the coagulation cascade. Crit Care Med. 1992; 20(10):1402–1405.

28. Ruzicka J, Stengl M, Bolek L, Benes J, Matejovic M, Krouzecky A. Hypothermic anticoagulation: testing individual responses to graded severe hypothermia with thromboelastography. Blood Coagul Fibrinolysis. 2012;23(4):285–289.

29. Sharma P, Saxena R. A novel thromboelastographic score to identify overt disseminated intravascular coagulation resulting in a hypocoagulable state. Am J Clin Pathol. 2010;134(1):97–102.

30. Soslau G, Prest PJ, Class R, George R, Paladino F, Violetta G. Comparison of sea turtle thrombocyte aggregation to human platelet aggregation in whole blood. Comp Biochem Physiol B Biochem Mol Biol. 2005;142(3):353–360.

31. Soslau G, Wallace B, Vicente C, Goldenberg SJ, Tupis T, Spotila J, George R, Paladino F, Whitaker B, Violetta G and others. Comparison of functional aspects of the coagulation cascade in human and sea turtle plasmas. Comp Biochem Physiol B Biochem Mol Biol. 2004;138(4):399–406.

32. Turnbull BS, Smith C, Stamper M. Medical implications of hypothermia in threatened loggerhead (*Caretta caretta*) and endangered Kemp's ridley (*Lepidochelys kempi*) and green (*Chelonia mydas*) sea turtles. 2000. AAZV; 1998. p. 31–35.

33. Valeri CR, Khuri GCS, Feingold H, Mark GR. Hypothermia-induced reversible platelet dysfunction. Ann Surg. 1987;205(2):175–181.

34. Valeri CR, MacGregor H, Cassidy G, Tinney R, Pompei F. Effects of temperature on bleeding time and clotting time in normal male and female volunteers. Crit Care Med. 1995;23(4):698–704.

35. Watts DD, Trask A, Soeken K, Perdue P, Dols S, Kaufmann C. Hypothermic coagulopathy in trauma: effect of varying levels of hypothermia on enzyme speed, platelet function, and fibrinolytic activity. J Trauma. 1998;44(5):846–854.

36. Wiinberg B, Kristensen AT. Thromboelastography in veterinary medicine. New York: Thieme Medical Publishers; 2010. p. 747–756.

37. Yoshihara H, Yamamoto T, Mihara H. Changes in coagulation and fibrinolysis occurring in dogs during hypothermia. Thromb Res. 1985;37(4):503–512.

Accepted for publication 3 November 2018