The following supplement accompanies the article

Clinicopathological prognostic indicators of survival and pathological findings in cold-stressed Florida manatees *Trichechus manatus latirostris*

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Table S1. Intake and pre-release blood analyte data in conventional units for survivor (n=49) cold-stressed syndrome Florida manatees (*Trichechus manatus latirostris*) admitted to a rehabilitation facility between 2007-2017. Significant values $P < 0.05$ indicated in grey background.

Blood analyte	Admission *Median (1st, 3rd quartile)*	Prerelease *Median (1st, 3rd quartile)*	p
Hemoglobin (g/dl)	12.4 (11.3, 13.7)	10.7 (10.1, 11.3)	<0.01
Hematocrit (%)	36.8 (33.3, 41.1)	31.7 (29.9, 33.8)	<0.01
RBC count (x^6/l)	3.10 (2.73, 3.32)	2.66 (2.51, 2.80)	<0.01
Mean corpuscular volume (fl)	118 (113, 123)	118 (115, 124)	0.17
Mean corpuscular hemoglobin (pg)	40.4 (38.8, 42.2)	40.3 (38.7, 42.2)	0.21
Mean corpuscular hemoglobin concentration (g/dl)	34.2 (33.7, 34.5)	33.9 (33.3, 34.3)	< 0.01
Red cell distribution width (%)	16.75 (16.02, 17.37)	16.35 (15.72, 17.37)	0.15
Platelet count ($x10^3$/µl)	406 (287, 530)	354 (301, 428)	0.02
Mean platelet volume (fl)	4.68 (4.04, 5.31)	4.48 (4.10, 4.74)	0.01
nRBC per 100 WBCs (%)	3 (0, 12)	0 (0, 1)	<0.01
nRBC (x^3/µl)	0.42 (0, 1.80)	0 (0, 0.02)	<0.01
Total WBC ($x10^3$/µl)	13.30 (9.00, 18.35)	7.49 (6.03, 8.60)	<0.01
Band heterophils ($x10^3$/µl)	0.46 (0.10, 1.33)	0.06 (0, 0.10)	<0.01
Heterophils ($x10^3$/µl)	8.19 (4.78, 10.91)	3.66 (3.13, 4.32)	<0.01
Lymphocytes ($x10^3$/µl)	2.16 (1.60, 3.28)	3.05 (1.97, 3.78)	0.02
Monocytes ($x10^3$/µl)	1.40 (0.70, 2.13)	0.47 (0.37, 0.68)	<0.01
Eosinophils ($x10^3$/µl)	0 (0, 0.18)	0 (0, 0.07)	0.18
Basophils ($x10^3$/µl)	0 (0, 0)	0 (0, 0.06)	0.07
Erythrocyte sedimentation rate (mm/hr)	55 (34, 80)	35 (22, 50)	<0.01
Glucose (mg/dl)	107 (89.5, 141)	66 (56, 73)	<0.01
BUN (mg/dl)	11 (9, 15)	12 (11, 15)	0.47
Creatinine (mg/dl)	1.3 (1.0, 1.5)	2.1 (1.6, 2.3)	<0.01
BUN:creatinine ratio	10 (6.67, 12.73)	6.36 (5.50, 7.86)	<0.01
Bilirubin (mg/dl)	0.1 (0.1, 0.1)	0.1 (0.1, 0.1)	0.75
Cholesterol (mg/dl)	315 (234, 350)	133 (121, 174)	<0.01
Triglyceride (mg/dl)	103 (63.5, 149.0)	72.5 (60.0, 91.2)	0.01

Blood analyte	Admission *Median (1st, 3rd quartile)*	Prerelease *Median (1st, 3rd quartile)*	p
Total protein (g/dl)	7.4 (7.1, 7.9)	7.4 (7.0, 7.9)	0.67
Albumin (g/dl)	3.9 (3.6, 4.3)	4.7 (4.5, 5.1)	<0.01
Globulin (g/dl)	3.4 (3.1, 4.1)	2.6 (2.4, 3.0)	<0.01
Albumin: Globulin ratio	1.15 (0.91, 1.37)	1.83 (1.55, 2.09)	<0.01
Alkaline phosphatase (U/l)	64 (53, 73)	89 (70, 105)	<0.01
Alanine aminotransferase (U/l)	8 (5, 14)	8 (6, 11)	0.42
Aspartate aminotransferase (U/l)	9 (7, 14)	6 (5, 7)	<0.01
Gamma-glutamyl transferase (U/l)	49 (41, 56)	40 (37, 44)	<0.01
Creatine kinase (U/l)	610 (387, 1563)	271 (160, 453)	<0.01
Lactate dehydrogenase (U/l)	554 (389, 951)	312 (247, 436)	<0.01
Calcium (mg/dl)	9.8 (9.4, 10.5)	10.4 (10.1, 10.8)	<0.01
Phosphorus (mg/dl)	5.9 (5.0, 7.0)	4.8 (4.2, 5.6)	<0.01
Calcium: phosphorus ratio	1.67 (1.40, 2.07)	2.21 (1.83, 2.48)	<0.01
Sodium (mEq/l)	142 (139, 144)	148 (146, 149)	<0.01
Potassium (mEq/l)	4.3 (4.0, 4.9)	4.5 (4.2, 4.7)	0.79
Sodium: potassium ratio	32.22 (27.96, 35.75)	32.44 (30.42, 35.61)	0.37
Chloride (mEq/l)	90 (87, 93)	93 (92, 97)	<0.01
Carbon dioxide (mEq/l)	37 (32, 42)	44 (38, 47)	<0.01
Iron (µg/dl)	42 (30, 60)	136 (103, 149)	<0.01
Fibrinogen (mg/dl)	276 (243, 289)	247 (228, 264)	0.04
Serum amyloid A (µg/ml)	170 (148.0, 191.0)	1 (0.5, 3)	<0.01

Table S2. Admission blood analyte data in conventional units of survivor (n= 49) and non-survivor (n=7) cold-stressed syndrome Florida manatees (*Trichechus manatus latirostris*) admitted to a rehabilitation facility between 2007-2017. Significant values $P < 0.05$ indicated in grey background.

Blood analyte	Survivors *Median (1st, 3rd quartile)*	Non-survivors *Median (1st, 3rd quartile)*	p
Hemoglobin (g/dl)	12.4 (11.3, 13.7)	11.3 (9.7, 12.8)	0.09
Hematocrit (%)	36.8 (33.3, 41.1)	33.9 (28.7, 36.4)	0.11
RBC (x^6/l)	3.10 (2.73, 3.32)	2.98 (2.33, 3.16)	0.20
Mean corpuscular volume (fl)	118 (113, 123)	118 (112, 121)	0.64
Mean corpuscular hemoglobin (pg)	40.4 (38.3, 42.2)	40.2 (38.1, 41.5)	0.60
Mean corpuscular hemoglobin concentration (g/dl)	34.2 (33.7, 34.6)	34.2 (33.5, 35.3)	0.69
Red cell distribution width (%)	16.7 (16.0, 17.4)	16.9 (16.7, 20.1)	0.05
Platelet count (x 10^3/µl)	399 (289, 522)	189 (75, 301)	< 0.01
Mean platelet volume (fl)	4.68 (4.05, 5.29)	4.55 (3.77, 6.53)	0.93
nRBC per 100 WBCs (%)	3.50 (0, 12.5)	26 (4.75, 81.25)	0.06
nRBC (x^3/µl)	0.42 (0, 1.79)	1.35 (0.12, 6.55)	0.20
Total WBC ($x10^3$/µl)	13.3 (9.0, 18.35)	11.1 (10.2, 13.0)	0.30
Band heterophils ($x10^3$/µl)	0.46 (0.10, 1.33)	1.84 (0, 2.34)	0.21

Blood analyte	Survivors *Median (1^{st}, 3^{rd} quartile)*	Non-survivors *Median (1^{st}, 3^{rd} quartile)*	p
Heterophils (x10^3/µl)	8.19 (4.78, 10.91)	6.76 (0.24, 8.44)	0.20
Lymphocytes (x10^3/µl)	2.16 (1.60, 3.28)	2.0 (1.22, 3.90)	0.76
Monocytes (x10^3/µl)	1.40 (0.70, 2.13)	1.46, (0.67, 1.95)	0.93
Eosinophils (x10^3/µl)	0 (0, 0.18)	0.18 (0.10, 0.24)	0.08
Basophils (x10^3/µl)	0 (0,0)	0 (0, 0)	1.00
Erythrocyte sedimentation rate (mm/hr)	56 (34, 80)	63 (35, 73)	0.78
Glucose (mg/dl)	107 (89, 141)	124 (83, 141)	1.00
BUN (mg/dl)	11 (9, 15)	22 (12, 28)	0.01
Creatinine (mg/dl)	1.3 (1.0, 1.5)	1.6 (1.1, 2.0)	0.14
BUN:creatinine ratio	10 (6.67, 13.15)	16.47 (11.50, 19.10)	0.07
Bilirubin (mg/dl)	0.1 (0.1, 0.1)	0.1 (0.1, 0.1)	0.25
Cholesterol (mg/dl)	315 (239, 349)	252 (207, 334)	0.37
Triglycerides (mg/dl)	103 (64, 149)	135 (65, 188)	0.44
Total protein (g/dl)	7.4 (7.1, 7.8)	6.4 (5.3, 7.7)	0.03
Albumin (g/dl)	3.9 (3.6, 4.3)	3.5 (2.5, 3.7)	0.05
Globulin (g/dl)	3.3 (3.1, 4.1)	3.1 (2.7, 3.6)	0.25
Albumin: globulin ratio	1.15 (0.91, 1.36)	1.19 (0.80, 1.30)	0.69
Alkaline phosphatase (U/l)	64 (52, 73)	64 (42, 122)	0.81
Alanine aminotransferase (U/l)	8 (5, 14)	10 (5, 12)	0.77
Aspartate aminotransferase (U/l)	9 (7, 13)	20 (14, 26)	<0.01
Gamma-glutamyl transferase (U/l)	49 (41, 55)	78 (39, 85)	0.08
Creatine kinase (U/l)	640 (388, 1548)	2744 (832, 4931)	0.03
Lactate dehydrogenase (U/l)	560 (389.5, 943.5)	827 (588, 1110)	0.06
Calcium (mg/dl)	9.8 (9.3, 10.5)	9.0 (8.5, 9.3)	<0.01
Phosphorus (mg/dl)	5.9 (5.1, 7.1)	5.9 (5.5, 8.1)	0.46
Calcium: phosphorus ratio	1.64 (1.39, 2.06)	1.44 (1.23, 1.6)	0.08
Sodium (mEq/l)	142 (139, 144)	133 (125, 145)	0.21
Potassium (mEq/l)	4.3 (4.05, 4.90)	5.2 (4, 5.9)	0.12
Sodium: potassium ratio	32.27 (28.11, 35.43)	27.89 (21.75, 33.25)	0.12
Chloride (mEq/l)	91 (87, 92.5)	85 (78, 98)	0.29
Carbon dioxide (mEq/l)	37 (32, 42)	33 (24,46)	0.26
Iron (µg/dl)	42 (29, 60)	22 (16, 53)	0.08
Fibrinogen (mg/dl)	262 (235, 289)	225 (158, 292)	0.19
Serum amyloid A (µg/ml)	170 (149, 191)	148 (145, 186)	0.32

Table S3. Cut-off points in conventional units, sensitivity, specificity, area under the curve (AUC), and confidence intervals (CI) for identification of cold-stressed syndrome Florida manatees *Trichechus manatus latirostris* that survived after admission to rehabilitation. Blood analytes with different superscripts (a,b) are different ($p < 0.05$).

Blood analyte	Cut-off point	Sensitivity	Specificity	Area Under Curve	95% CI
Platelets ($x10^3/\mu l$)	> 301	72.9	85.7	$0.810^{a,b}$	0.681-0.903
Aspartate aminotransferase (U/l)	≤ 12	75.5	85.7	$0.810^{a,b}$	0.684-0.903
Calcium (mg/dl)	> 9.3	75.5	85.7	$0.805^{a,b}$	0.677-0.898
Blood urea nitrogen (mg/dl)	≤ 20	95.9	71.4	0.778^{b}	0.647-0.878
Total protein (g/dl)	> 6.8	87.8	71.4	$0.749^{a,b}$	0.615-0.855
Creatine kinase (U/l)	≤ 1594	79.6	71.4	$0.743^{a,b}$	0.609-0.851
Nucleated red blood cells per 100 white blood cells (%)	≤ 35	93.5	50.0	$0.737^{a,b}$	0.597-0.850
Albumin (g/dl)	> 3.7	65.3	85.7	$0.723^{a,b}$	0.587-0.834
Blood urea nitrogen:creatinine ratio	≤ 11.25	65.3	85.7	0.711^{b}	0.484-0.938
Calcium:phosphorus ratio	> 1.6	55.1	85.7	$0.706^{a,b}$	0.537-0.874
Iron (µg/dl)	> 29	75.0	71.4	$0.704^{a,b}$	0.565-0.819
Gamma-glutamyl transferase (U/l)	≤ 56	87.8	71.4	$0.701^{a,b}$	0.564-0.816
Serum amyloid A (µg/ml)	> 150	72.1	66.7	0.626^{a}	0.476-0.760

Table S4. Positive and negative predictive values for classification of cold-stress syndrome Florida manatees as survivors (yes, no). SI units

	90% prevalence*	95% prevalence*
Platelet count (n=100) Cut-off point ≥ 301 x 10^9/l Sensitivity = 72%; Specificity = 85% Positive predictive value % (95% CI) Negative predictive value % (95% CI)	 97.7 (90.7, 99.8) 25.2 (11.9, 43.1)	 98.9 (92.8, 100.0) 13.7 (4.1, 31.0)
AST, Ca (n=100) Cut-off point AST ≤ 0.20 µkat/l, Ca ≥ 2.33 mmol/l Sensitivity = 75%; Specificity = 85% Positive predictive value % (95% CI) Negative predictive value % (95% CI)	 97.3 (91.0, 99.8) 27.4 (13.0, 46.3)	 98.6 (93.1, 100.0) 15.1 (4.5, 33.7)
BUN (n=100) Cut-off point ≤ 7.14 mmol/l Sensitivity = 95%; Specificity = 71% Positive predictive value % (95% CI) Negative predictive value % (95% CI)	 96.7 (90.6, 99.3) 61.2 (29.6, 87.1)	 98.4 (93.3, 99.9) 42.7 (11.8, 78.8)

*Expected proportion of manatees with cold stress that survive after medical care = 90% or 95%.

Table S5. Positive and negative predictive values for classification of cold-stress syndrome Florida manatees as survivors (yes, no). Conventional units

	90% prevalence*	95% prevalence*
Platelet count (n=100) Cut-off point ≥ 301 x 10^3/µl Sensitivity = 72%; Specificity = 85% Positive predictive value % (95% CI) Negative predictive value % (95% CI)	 97.7 (90.7, 99.8) 25.2 (11.9, 43.1)	 98.9 (92.8, 100.0) 13.7 (4.1, 31.0)
AST, Ca (n=100) Cut-off point AST ≤ 12 U/l, Ca ≥ 9.3 mg/dl Sensitivity = 75%; Specificity = 85% Positive predictive value % (95% CI) Negative predictive value % (95% CI)	 97.3 (91.0, 99.8) 27.4 (13.0, 46.3)	 98.6 (93.1, 100.0) 15.1 (4.5, 33.7)
BUN (n=100) Cut-off point ≤ 20 mg/dl Sensitivity = 95%; Specificity = 71% Positive predictive value % (95% CI) Negative predictive value % (95% CI)	 96.7 (90.6, 99.3) 61.2 (29.6, 87.1)	 98.4 (93.3, 99.9) 42.7 (11.8, 78.8)

*Expected proportion of manatees with cold stress that survive after medical care = 90% or 95%.

Table S6. Gross and histopathological findings in non-surviving cold-stress syndrome Florida manatees presented to SeaWorld Orlando for rehabilitation between 2007-2017. na = not applicable

Pathological findings	Gross	Histopathology
Cutaneous lesions (epidermal ulceration, bleaching, hyperplasia, or dermatitis)	14	11
Lipid depletion	12	3
Pancreatic zymogen depletion due to starvation	na	7
Gastrointestinal hemorrhage or ulceration	9	6
Stomach mucosal necrosis	na	3
Pneumonia	na	5
Pulmonary edema	10	3
Pleural effusion	1	na
Pneumoconiosis	na	1
Skeletal muscle pathological findings (necrosis, abscess, or myositis)	4	2
Enteritis	na	3
Vacuolar hepatopathy	na	2
Hepatic congestion	4	1
Keratitis	na	1
Hepatitis	na	2
Hepatic glycogenosis	na	1
Bronchitis	na	1
Tracheitis	na	1
Nasopharyngitis	na	1
Lung abscesses	2	1
Intravascular thrombosis (lung or dermal)	na	7
Renal pathological findings (tubuloproteinosis or interstitial fibrosis)	na	2
Swollen Kidneys (gross) or renal congestion (histopathological)	2	2
Adrenal enlargement	1	na
Myocardial edema	na	1
Myocardial atrophy	na	1
Cerebral meningeal congestion/edema	1	5
Pancreatic hemorrhage	1	
Splenic and/or Extramedullary hematopoeisis	na	1
Splenic plasmacytosis	na	1
Lymphoid depletion (T-septal lymph node or spleen)	na	2
Enlarged Lymph nodes (gross) or lymphoid hyperplasia (histopathological)	4	1
Ascites	2	na